REPORT

Study Title:

Hydrolysis of K32 in pH 4, pH 7 and pH 9 Buffered Water

Ricerca Document Number: 035234-1

Study Completed:
13-June-2017

Author:
Penny Miner

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study reported herein, "Hydrolysis of K32 in pH 4, pH 7 and pH 9 Buffered Water" Ricerca Biosciences, LLC Study Number 035234 was conducted and reported in compliance with the Good Laboratory Practice Regulations set forth in Title 40, Part 160 of the Code of Federal Regulations of the United States of America.

Penny Miner — June 13, 2017

Penny Miner, Study Director
AgChem Product Development
Ricerca Biosciences, LLC

Date

Eric C. Searcy — 6/13/2017

Eric Searcy, Sponsor Representative
Koch Agronomic Services, LLC

Date

Eric C. Searcy — 6/13/2017

Submitter

Date

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10 (d) (1) (A), (B), or (C).

Company: Koch Agronomic Services

Company Agent: Eric A. Searcy

Title: Product Regulatory Manager

Eric A. Searcy
Signature

6/13/207
Date

These data are the property of Koch Agronomic Services, LLC, and, as such, are considered confidential for all purposes other than compliance with FIFRA Section 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other statute or in any other country.

QUALITY ASSURANCE STATEMENT

The Ricerca Quality Assurance Unit has performed inspections on the study, "Hydrolysis of K32 in pH 4, pH 7 and pH 9 Buffered Water" Ricerca Biosciences, LLC Study Number 035234. The results of these inspections, including any findings or observations, were reported to the Study Director and Management for appropriate corrective actions on the dates listed below.

Phase Inspected	Date of Inspection	Dates Reported to the Study Director	Dates Reported to Management
Protocol	December 13, 2016	December 13, 2016	December 13, 2016
In-Study	January 31, 2017	January 31, 2017	January 31, 2017
Data/Report	May 24 & 25, 2017	May 25, 2017	May 25, 2017

Ann L. O'Leary, Ph.D.
Ricerca Biosciences, LLC Quality Assurance

June 13, 2017
Date

APPROVALS

Study Title: Hydrolysis of K32 in pH 4, pH 7 and pH 9 Buffered Water

Document Number: 035234-1

Testing Facility: Ricerca Biosciences, LLC
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Penny Miner
Penny Miner, Study Director
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6/13/17
Date

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SUMMARY

The objective of this study was to determine the rate of hydrolysis of K32 in sterile pH 4.0, 7.0, and 9.0 buffers for 5 Days. The product ("K32") is a complex mixture of molecules which are designed to break down into smaller components of known toxicological and environmental fate (e.g., NBPT and UF derivatives); therefore, only a Tier-0 hydrolysis of "K32" was completed. "K32" was shown to transform into NBPT under hydrolyzing conditions.

A half-saturated test solution of K32 was incubated at 50 °C for 5 days in sterile pH 4.0, 7.0, and 9.0 buffers. K32 test solution was analyzed prior to dilution with buffers (time = -1 Day). The test solution was sampled and analyzed on Days 0, 1, 3, and 5 to confirm the pH. Concurrently, these samples were assayed by LC/MS with UV at each sampling interval to determine the distribution of components.

Component	Observed Retention Time (minutes)	Expected Molecular Mass	Observed molecular mass
1	6.58	478 and 406 [M+Na]+	406.1 [M+Na]+
2	6.98	334 [M+Na]+	334.1 [M+Na]+
3a	7.48	262 [M+Na]+	262.1 [M+Na]+
3b	7.76		334.1 [M+Na]+
4	10.83	406 [M+Na]+	406.1 [M+Na]+
5	11.06	334 [M+Na]+	334.1 [M+Na]+
6	11.48	262 [M+Na]+	262.1 [M+Na]+
7	12.16	190 [M+Na]+	168.1 [M+H]+
8	12.62	406 [M+Na]+	406.1 [M+Na]+
9	12.89	334 [M+Na]+	334.1 [M+Na]+
11	17.4 to 30.0	Not Defined, mixture	Not Defined, mixture

Sample pH increased with time between Days 0 and 5 for the pH 4 buffer. Sample pH for the pH 7 buffer remained consistent. The pH for the samples in pH 9 buffer decreased from 9.00 at time zero to 8.21 on Day 5.

Peak areas for each component versus time are summarized in the tables below.

pH 4 Buffer						
Peak No.	T=-1*	T = 0	T=1	T=3	T=5	MRM amu
1	126.70	ND	ND	ND	ND	406.10/274.10 amu
2	984.81	ND	ND	ND	ND	334.10/189.10 amu
3	2126.38	ND	ND	ND	ND	262.20/83.11 amu (3a) 334.10/155.10 amu (3b)
4	1512.82	66.90	9.25	ND	ND	334.10/274.10 amu
5	8392.05	243.32	18.30	ND	ND	262.20/147.10 amu
6	15028.00	4442.21	2132.51	245.78	26.41	168.10/95.10 amu (NBPT)
7	304.25	64.30	7.39	3.07	1.30	406.10/274.10 amu
8	312.31	4.25	0.06	ND	ND	334.10/124.10 amu
9	18177.72	915.08	1225.20	1547.07	1563.01	Not defined

*T=-1 K32 is dissolved in acetonitrile:water and analyzed by LC/MS prior to buffer addition.

pH 7 Buffer						
Peak No.	T=-1*	T = 0	T=1	T=3	T=5	MRM amu
1	126.70	53.13	170.48	156.12	83.72	406.10/274.10 amu
2	984.81	1065.78	1044.78	922.84	659.55	334.10/189.10 amu
3	2126.38	2440.26	2456.17	1796.22	934.59	262.20/83.11 amu (3a) 334.10/155.10 amu (3b)
4	1512.82	1477.16	1586.38	2101.76	2105.72	334.10/274.10 amu
5	8392.05	8909.57	10021.00	12785.00	13220.00	262.20/147.10 amu
6	15028.00	15020.00	20588.00	30901.00	34691.00	168.10/95.10 amu (NBPT)
7	304.25	306.42	812.79	2170.40	2588.38	406.10/274.10 amu
8	312.31	177.65	192.36	238.53	250.71	334.10/124.10 amu
9	18177.72	18635.39	16269.38	11876.59	8536.81	Not defined

*T=-1 K32 is dissolved in acetonitrile:water and analyzed by LC/MS prior to buffer addition.

pH 9 Buffer						
Peak No.	T=-1*	T = 0	T=1	T=3	T=5	MRM amu
1	126.70	135.50	174.72	209.09	190.64	406.10/274.10 amu
2	984.81	1027.40	1105.16	1200.55	1166.03	334.10/189.10 amu
3	2126.38	2388.87	2572.91	2943.28	2761.44	262.20/83.11 amu (3a) 334.10/155.10 amu (3b)
4	1512.82	1475.35	1562.08	1746.55	1778.92	334.10/274.10 amu
5	8392.05	8822.66	9442.07	11160.00	11541.00	262.20/147.10 amu
6	15028.00	15579.00	17445.00	23587.00	26651.00	168.10/95.10 amu (NBPT)
7	304.25	151.68	386.03	978.17	1265.17	406.10/274.10 amu
8	312.31	32.83	180.33	212.09	216.97	334.10/124.10 amu
9	18177.72	19033.96	18097.59	16455.28	13022.67	Not defined

*T=-1 K32 is dissolved in acetonitrile:water and analyzed by LC/MS prior to buffer addition.

The concentrations of all components in all buffers decreased with time except for the NBPT which increased in time for the pH 7 and pH 9 buffers.

INTRODUCTION

The objective of this study was to determine the rate of hydrolysis of K32 in sterile pH 4.0, 7.0, and 9.0 buffers.

STUDY INFORMATION

STUDY NUMBER

035234

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SCHEDULE OF EVENTS

Study Initiation Date:	January 11, 2017
Experimental Start Date:	January 27, 2017
Experimental Termination Date:	February 28, 2017

REGULATORY COMPLIANCE

This study was conducted following registration requirements in accordance with U.S. EPA Fate, Transport and Transformation Test Guidelines OPPTS 835.2120-Hydrolysis, (adopted October 2008). This study was conducted according to the U.S. EPA Good Laboratory Practice Standards, 40 CFR Part 160.

RETENTION OF DATA

Records, which include at a minimum all of the raw data, the original signed protocol and any amendments thereto, letters, memos or notes, which pertain to the study and the original signed report, will be temporarily maintained by the Testing Facility until the Sponsor approves their transfer to a designated permanent archive. All non-study specific raw data (e.g., instrument logs and facility records) shall be archived at Ricerca Biosciences, LLC.

MATERIALS AND METHODS

TEST SUBSTANCE

- K32

Composition:	Reaction products of NBPT with urea and formaldehyde
Batch/Lot Number:	55700-30-13
Analyzed Concentration:	NBPT 20.04 wt%
Manufactured by:	Ricerca Biosciences
Date of manufacture:	July 20, 2016
Appearance:	Off-white to pale yellow gel
Storage:	Refrigerated

ANALYTICAL PROCEDURES

The peak areas of components in hydrolysis study samples were determined by liquid chromatography with ultraviolet detection (LC/UV). The identities of components in the test substance were confirmed by LC-MS/MS analytical method that utilized a very specific and sensitive Multiple Reaction Monitoring (MRM) method.

All LC-MS data were collected by Analyst®. Microsoft Excel™ (non-validated software) was used as to generate necessary statistics.

PREPARATION OF K32 STUDY SAMPLE PRIOR TO BUFFER (T=-1 DAY)

K32 (~2.65 g) was weighed into a beaker with a new stir bar. Acetonitrile, 150 mL was added to dissolve the solid K32 then 350 mL of distilled water was added (3:7 ACN:Water,v/v). The beaker was loosely covered and placed on stir plate and stirred for several hours at room temperature until the solution appeared homogeneous. A 2-mL subsample was removed from the beaker (Day -1 sample) and placed in a glass vial. The subsample was analyzed freshly on the day of removal of the subsample from the main sample.

PREPARATION OF HYDROLYSIS STUDY SAMPLES

K32 (~5.30 g) was weighed into three sterilized beaker with a new stir bar. Acetonitrile, 300 mL was added to dissolve the solid K32 then 700 mL of distilled water was added (3:7 ACN:Water, v/v). The beaker was loosely covered and placed on stir plate and stirred for several hours at room temperature until the solution appeared homogeneous. The pH of each beaker was taken. To separate beakers, pH 4, 7, or 9 buffers were added until the half-saturated stock solution from above matched the intended buffer pH, therefore creating a K32 solution in pH 4, 7, and 9.

A 2-mL subsample was removed from the beaker immediately after dilution (Day 0 sample) and placed in a glass vial. The remaining sample was placed in an oven set at 50 ºC. On

Days 1, 3, and 5, 10-mL subsamples were removed from the main sample. Each subsample was analyzed.

The pH of each subsample was measured using a Fisher Accumet AB150 pH meter. Then, a 1-mL aliquot of each subsample was removed for LC-MS analysis.

LC-UV AND LC-MS-MRM ANALYSIS OF HYDROLYSIS STUDY SAMPLES

The UV peak area of each component was obtained. Identification of components in each hydrolysis study sample was conducted by Multiple Reaction Monitoring (MRM) method of the following components:

Component	Approximate Retention Time	MRM+
1	6.73	406.10/274.10 amu
2	7.11	334.10/189.10 amu
3	7.58	262.20/83.11 amu
3b	8.06	334.10/155.10 amu
4	11.34	334.10/274.10 amu
5	11.79	262.20/147.10 amu
6	12.50	168.10/95.10 amu (NBPT)
7	13.05	406.10/274.10 amu
8	13.42	334.10/124.10 amu
9	14.27-31.91	Not defined

A solution of K32 was infused into the mass spectrometer for (+)Q1 scan. The mass spectrometer was tuned to achieve maximum response using positive ionization mode. See the tables below for a summary of mass spec methods and parameters.

LC-MS/MS Method for Positive Ionization Mode

Liquid Chromatograph	Shimadzu Shimadzu LC-20A Pumps and SIL-30ACMP Autosampler
Analytical column	Restek Ultra C18, 5µm, 150 x 4.6 mm
Flow rate	0.9 ml/min
Temperature	30° C
Pumping Mode	Gradient
Mobile phase A	Water
Mobile phase B	Acetontrile

Time (minutes)	%A	%B
Initial	87	13
8.00	87	13
30.00	30	70
31.00	87	13
35.00	87	13

MS/MS Method

Mass Spectrometer:	API-4000 Triple Quadrupole Mass Spectrometer (AB Sciex)
Data System:	Analyst 1.6.2
Ionization Mode:	Positive Ion Mode
Ion Spray Voltage (IS):	5500 V
Temperature (TEM):	600 °C
Declustering Potential (DP):	70
Curtain Gas (N_2) (CUR):	30 psi
Gas 1 (N_2):	60 psi
Gas 2 (N_2):	60 psi
Entrance Potential:	10 V
Collision Cell Exit Potential (CXP):	14 V
Scan Type:	MRM

The precursor masses from Q1 scans in positive ionization mode were found to be consistent with the correct theoretical nominal mass for all the components of K32.

Analysis of K32 Components

Component	Approximate Retention Time (minutes)	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision Energy (eV)
1	6.73	406.1	274.1	25
2	7.11	334.1	189.1	20
3	7.58	262.2	83.1	30
3b	8.06	334.1	155.1	20
4	11.34	334.1	274.1	25
5	11.79	262.2	147.1	25
6	12.50 (NBPT)	168.10	95.1	25
7	13.05	406.1	274.1	25
8	13.42	334.1	124.1	25

SIGNIFICANT FIGURES AND ROUNDING OF NUMBERS

No general rule was applied for significant figures. Due to the general analytical methodology used in the study, values presented with more significant figures do not imply that these values are more precise. For entry into a spreadsheet program or calculator, either instrumentally obtained values were entered as obtained or whole rounded integers were used for calculations. Data in tables are shown as rounded numbers for presentation purposes, but in the internal spreadsheet program or calculation the values were not rounded. Therefore, hand calculations to obtain values given in the tables of this report may differ slightly from those presented due to rounding and/or truncation.

RESULTS AND DISCUSSION

DETERMINATION OF CONTENT OF COMPONENTS IN HYDROLYSIS SAMPLES BY LC/UV ANALYSIS

The UV peak areas of each of the components were measured and compared to K32 prior to the addition of buffer in order to determine the time course of hydrolysis.

For each hydrolysis study sample, the sample pH was measured, the LC/UV peak areas of each component were obtained, and the identity of each component was confirmed by MRM mass spectrum.

Representative LC-UV chromatograms and mass spectra of the components are summarized in the table below.

Sample	Figure Number for LC/UV Chromatogram	Figure Number for Mass Spectra
ACN/ Water T= -1	Figure 1	Figure 2
pH 4 T= 0	Figure 3	Figure 4
pH 4 T= 3	Figure 5	Figure 6
pH 4 T=5	Figure 7	Figure 8
pH 7 T=0	Figure 9	Figure 10
pH 7 T=3	Figure 11	Figure 12
pH 7 T=5	Figure 13	Figure 14
pH 9 T=0	Figure 15	Figure 16
pH 9 T=3	Figure 17	Figure 18
pH 9 T=5	Figure 19	Figure 20

The concentrations of all components in all buffers decreased with time except for the NBPT which increased in time for the pH 7 and pH 9 buffers (Figure 21 through Figure 23).

MASS SPECTROMETRIC IDENTIFICATION OF PEAKS IN HYDROLYSIS STUDY SAMPLES

Identification of components in each hydrolysis study sample was conducted by obtaining the LC/UV retention time and were confirmed by LC-MS/MS analytical method, Multiple Reaction Monitoring (MRM).

CONCLUSIONS

The objective of this study was to determine the rate of hydrolysis of K32 in sterile pH 4.0, 7.0, and 9.0 buffers at 50º C for 5 days.

Sample pH increased with time between Days 0 and 5 for the pH 4 buffer. Sample pH for the pH 7 buffer remained consistent. The pH for the samples in pH 9 buffer decreased from 9.00 at time zero to 8.21 on Day 5.

The concentrations of all components in all buffers decreased with time except for the NBPT which increased over time for the pH 7 and pH 9 buffers.

PROTOCOL DEVIATION

Per the protocol, the test systems will be incubated in the dark at 50 ± 0.5 °C; however, on multiple occasions the temperature went out of the range of 49.5 to 50.5 °C for brief periods of time. This deviation in temperature bears no impact on the study as these occurrences were short in duration.

Table 1: Sample pH Versus Time

Day	Sample		
	pH 4	pH 7	pH 9
0	4.02	7.00	9.00
1	4.77	7.06	8.70
2	4.94	6.96	8.34
3	5.10	7.03	8.21

Table 2: Component Peak Areas Versus Time for pH 4 Buffer

pH 4 Buffer						
Peak No.	T=-1*	T = 0	T=1	T=3	T=5	MRM amu
1	126.70	ND	ND	ND	ND	406.10/274.10 amu
2	984.81	ND	ND	ND	ND	334.10/189.10 amu
3	2126.38	ND	ND	ND	ND	262.20/83.11 amu (3a) 334.10/155.10 amu (3b)
4	1512.82	66.90	9.25	ND	ND	334.10/274.10 amu
5	8392.05	243.32	18.30	ND	ND	262.20/147.10 amu
6	15028.00	4442.21	2132.51	245.78	26.41	168.10/95.10 amu (NBPT)
7	304.25	64.30	7.39	3.07	1.30	406.10/274.10 amu
8	312.31	4.25	0.06	ND	ND	334.10/124.10 amu
9	18177.72	915.08	1225.20	1547.07	1563.01	Not defined

*T=-1 K32 is dissolved in acetonitrile:water and analyzed by LC/MS prior to buffer addition.

Table 3: Component Peak Areas Versus Time for pH 7 Buffer

pH 7 Buffer						
Peak No.	T=-1*	T = 0	T=1	T=3	T=5	MRM amu
1	126.70	53.13	170.48	156.12	83.72	406.10/274.10 amu
2	984.81	1065.78	1044.78	922.84	659.55	334.10/189.10 amu
3	2126.38	2440.26	2456.17	1796.22	934.59	262.20/83.11 amu (3a) 334.10/155.10 amu (3b)
4	1512.82	1477.16	1586.38	2101.76	2105.72	334.10/274.10 amu
5	8392.05	8909.57	10021.00	12785.00	13220.00	262.20/147.10 amu
6	15028.00	15020.00	20588.00	30901.00	34691.00	168.10/95.10 amu (NBPT)
7	304.25	306.42	812.79	2170.40	2588.38	406.10/274.10 amu
8	312.31	177.65	192.36	238.53	250.71	334.10/124.10 amu
9	18177.72	18635.39	16269.38	11876.59	8536.81	Not defined

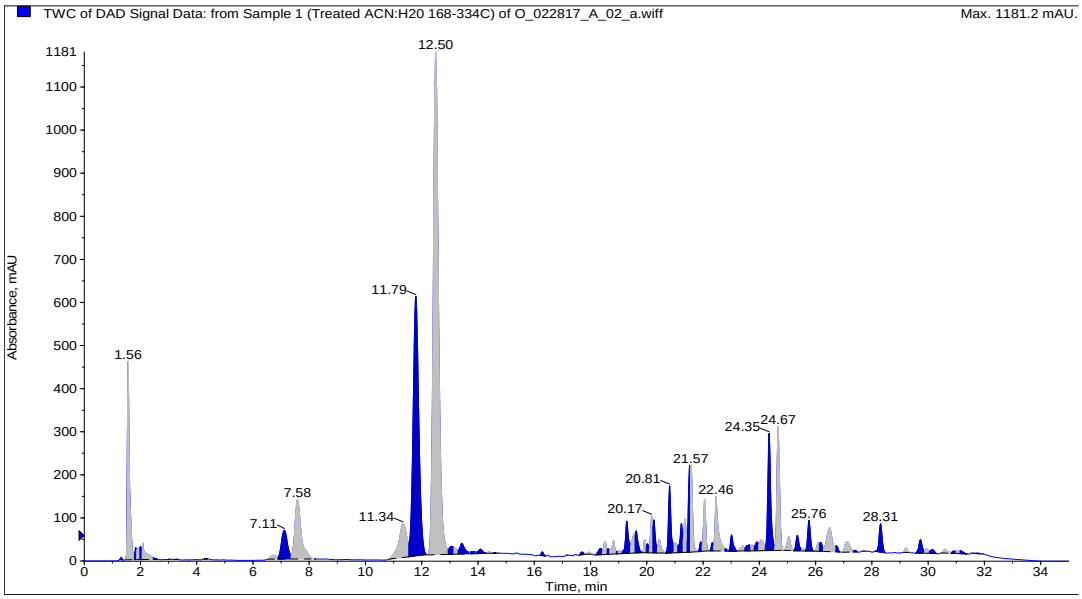
*T=-1 K32 is dissolved in acetonitrile:water and analyzed by LC/MS prior to buffer addition.

Table 4: Component Peak Areas Versus Time for pH 9 Buffer

pH 9 Buffer						
Peak No.	T=-1*	T = 0	T=1	T=3	T=5	MRM amu
1	126.70	135.50	174.72	209.09	190.64	406.10/274.10 amu
2	984.81	1027.40	1105.16	1200.55	1166.03	334.10/189.10 amu
3	2126.38	2388.87	2572.91	2943.28	2761.44	262.20/83.11 amu (3a) 334.10/155.10 amu (3b)
4	1512.82	1475.35	1562.08	1746.55	1778.92	334.10/274.10 amu
5	8392.05	8822.66	9442.07	11160.00	11541.00	262.20/147.10 amu
6	15028.00	15579.00	17445.00	23587.00	26651.00	168.10/95.10 amu (NBPT)
7	304.25	151.68	386.03	978.17	1265.17	406.10/274.10 amu
8	312.31	32.83	180.33	212.09	216.97	334.10/124.10 amu
9	18177.72	19033.96	18097.59	16455.28	13022.67	Not defined

*T=-1 K32 is dissolved in acetonitrile:water and analyzed by LC/MS prior to buffer addition.

Figure 1: Representative LC-MS/UV Chromatogram of Acetonitrile:Water K32 Sample, Day -1



Expanded 6-18 minutes

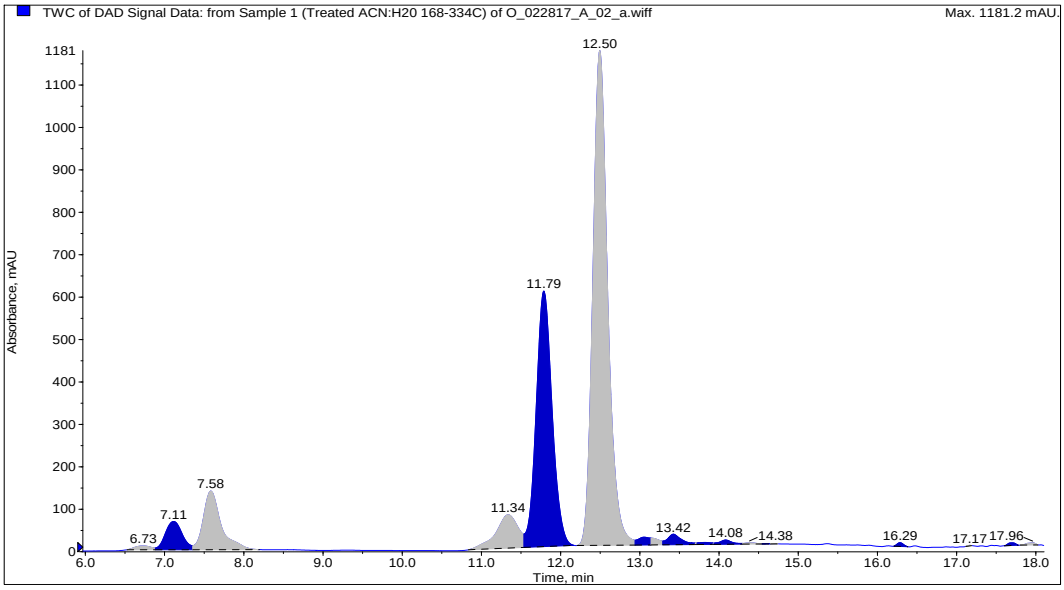


Figure 2: Mass Spectral Analysis for Acetonitrile:Water K32 Sample (MRM Analysis) from Hydrolysis Sample, Day -1

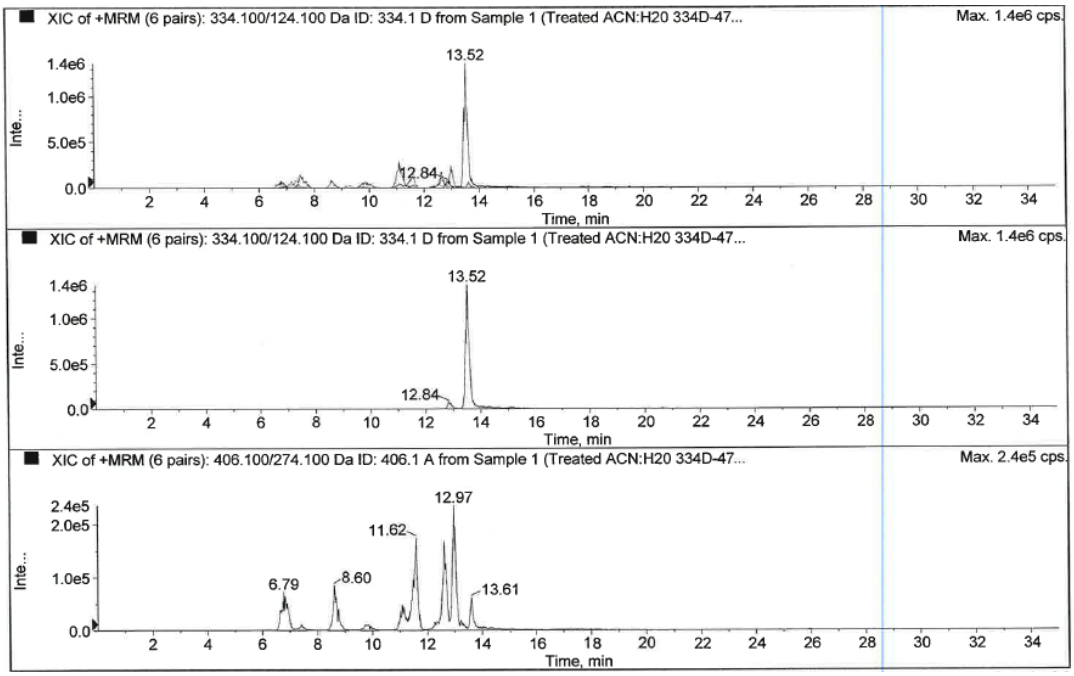


Figure 2: Mass Spectral Analysis for Acetonitrile:Water K32 Sample (MRM Analysis) from Hydrolysis Sample, Day -1 continued

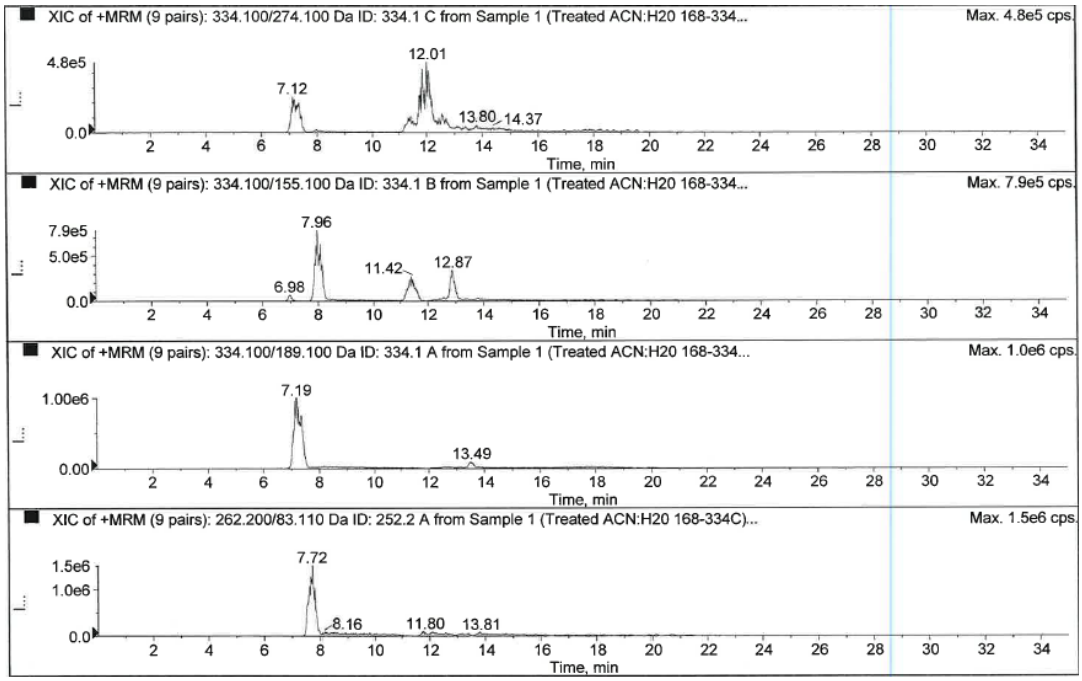


Figure 2: Mass Spectral Analysis for Acetonitrile:Water K32 Sample (MRM Analysis) from Hydrolysis Sample, Day -1 continued

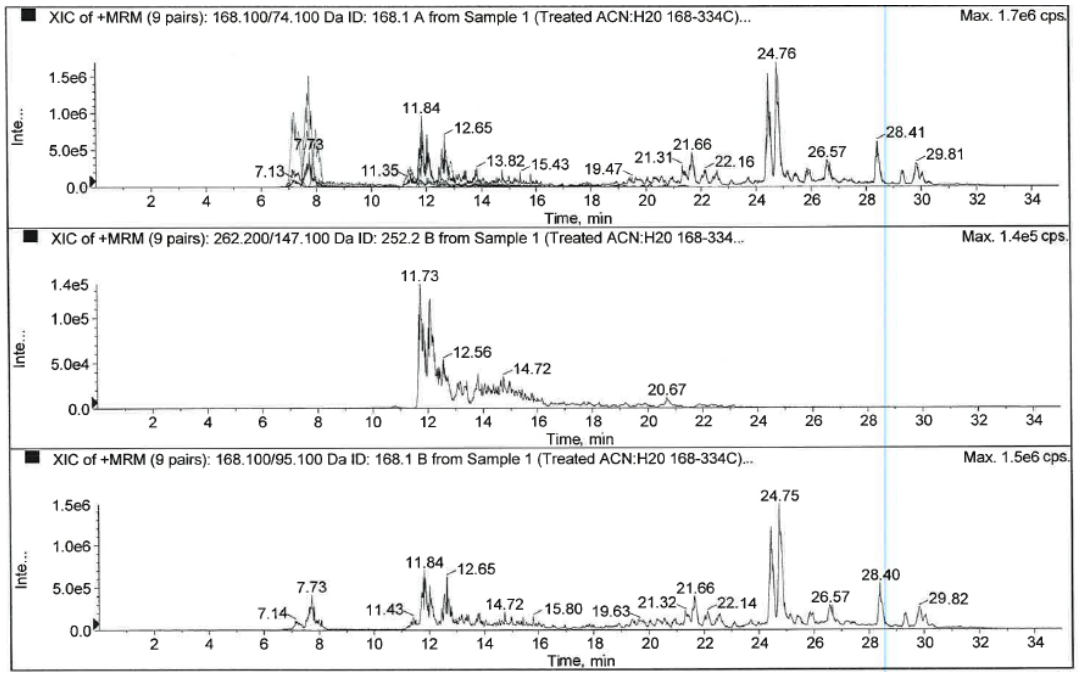
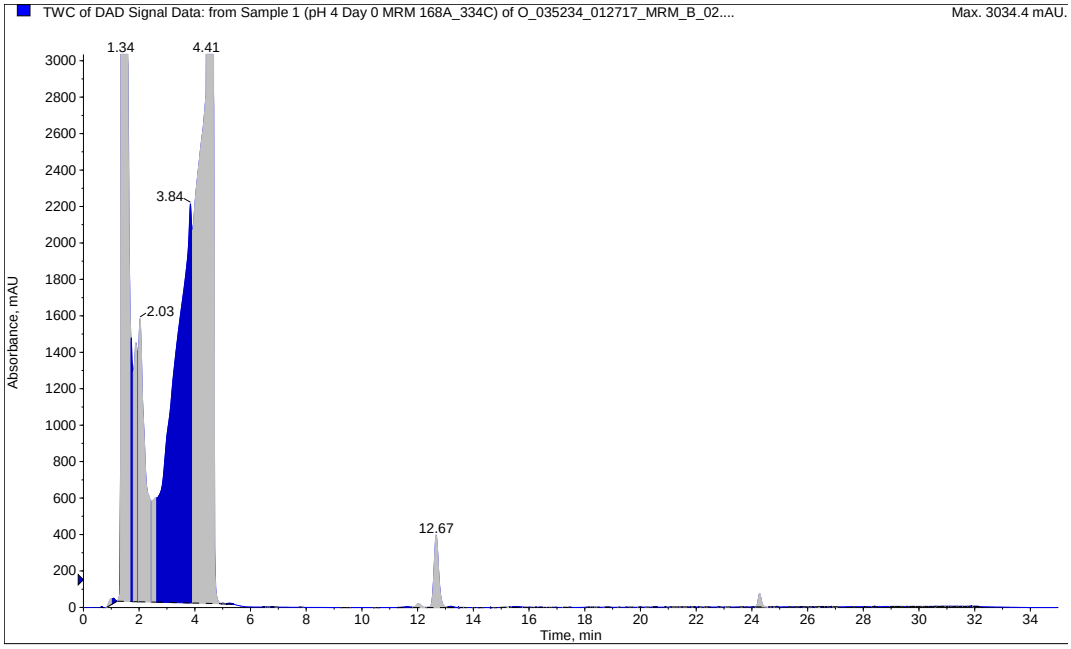


Figure 3: Representative LC-MS/UV Chromatogram of K32 Hydrolysis Sample in pH 4 Buffer, Day 0



Expanded 6-18 minutes

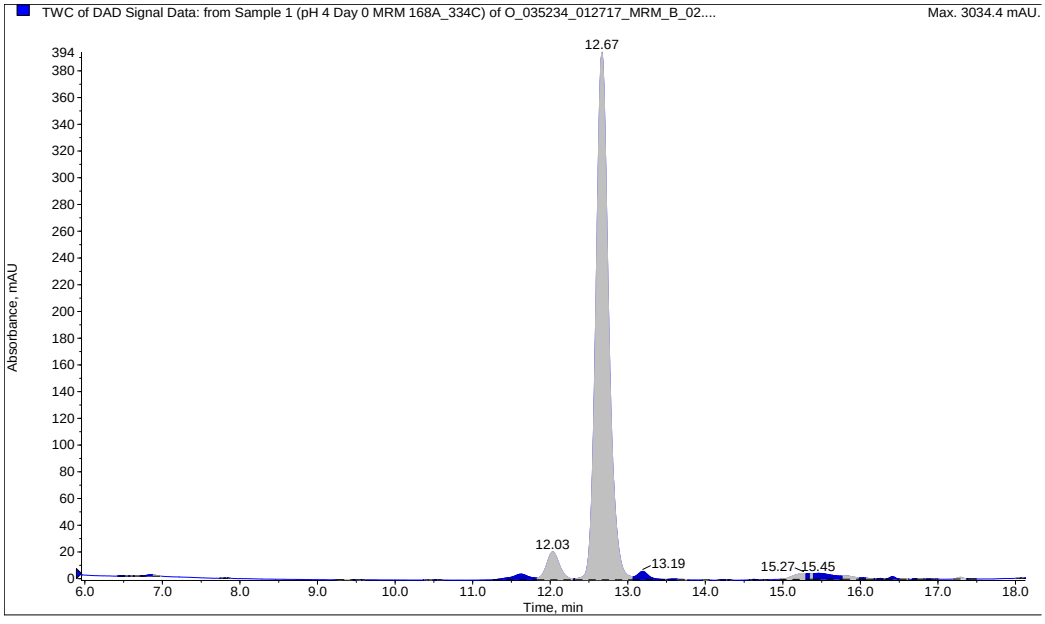


Figure 4: Mass Spectral Analysis for K32 Sample in pH 4 Buffer (MRM Analysis) from Hydrolysis Sample, Day 0

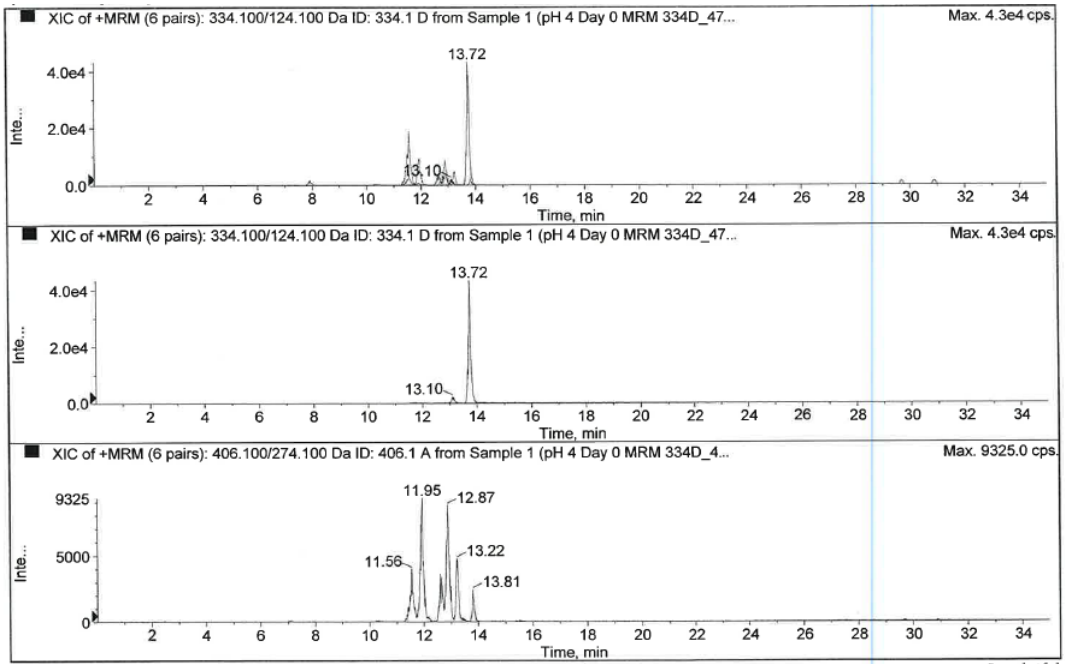


Figure 4: Mass Spectral Analysis for K32 Sample in pH 4 Buffer (MRM Analysis) from Hydrolysis Sample, Day 0 continued

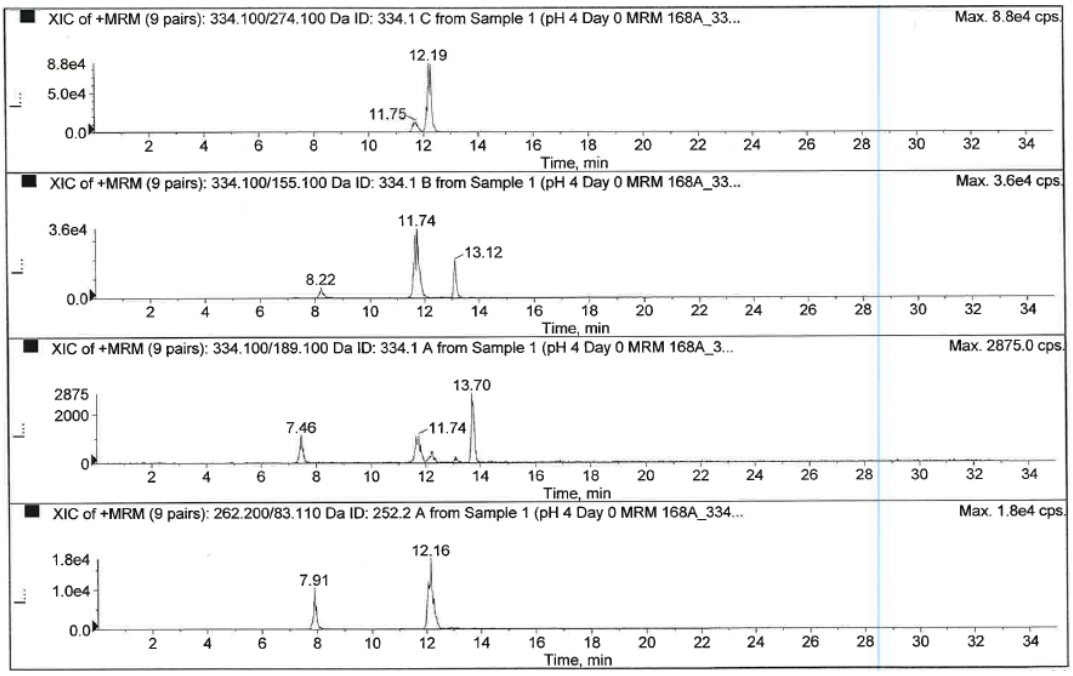


Figure 4: Mass Spectral Analysis for K32 Sample in pH 4 Buffer (MRM Analysis) from Hydrolysis Sample, Day 0 continued

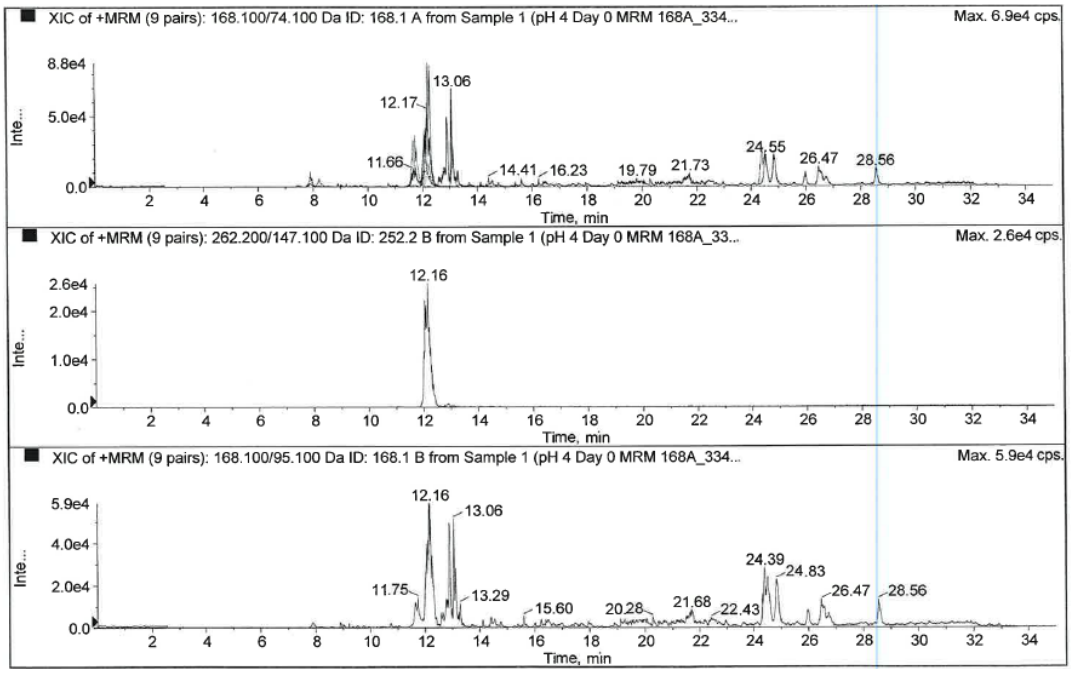
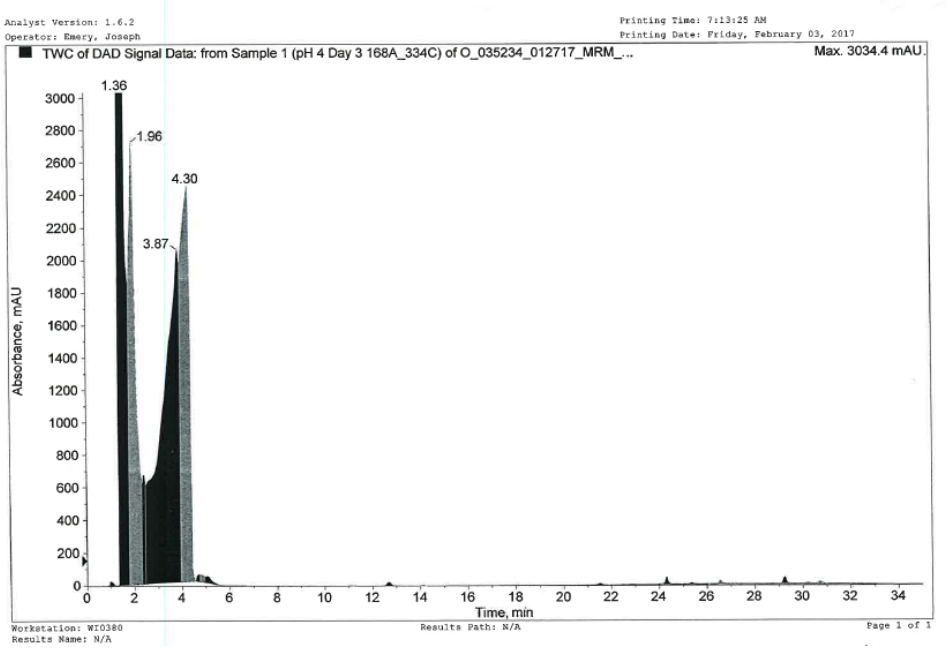


Figure 5: Representative LC-MS/UV Chromatogram of K32 Hydrolysis Sample in pH 4 Buffer, Day 3



Expanded 6-18 minutes

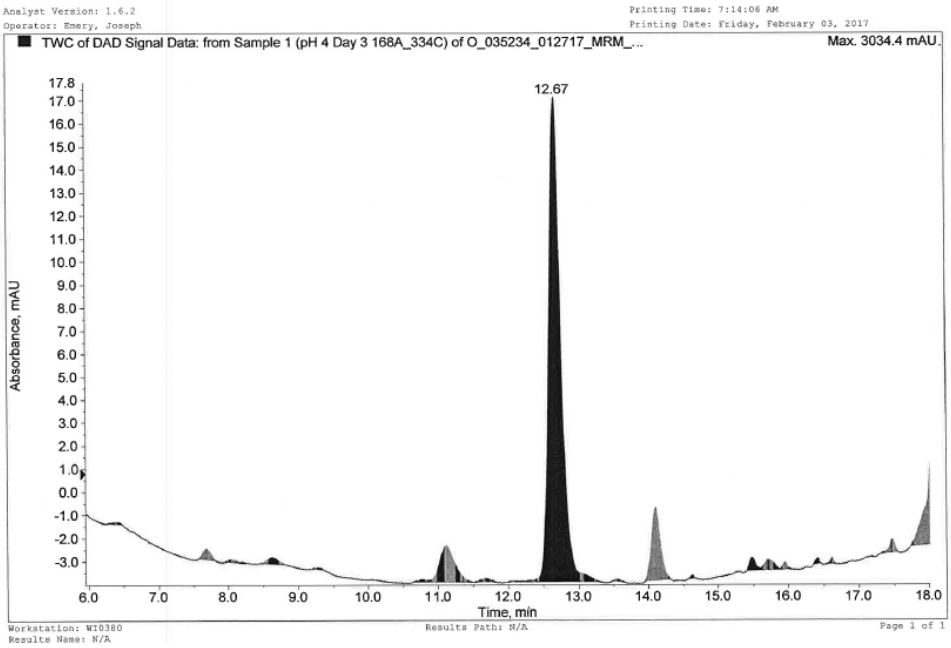


Figure 6: Mass Spectral Analysis for K32 Sample in pH 4 Buffer (MRM Analysis) from Hydrolysis Sample, Day 3

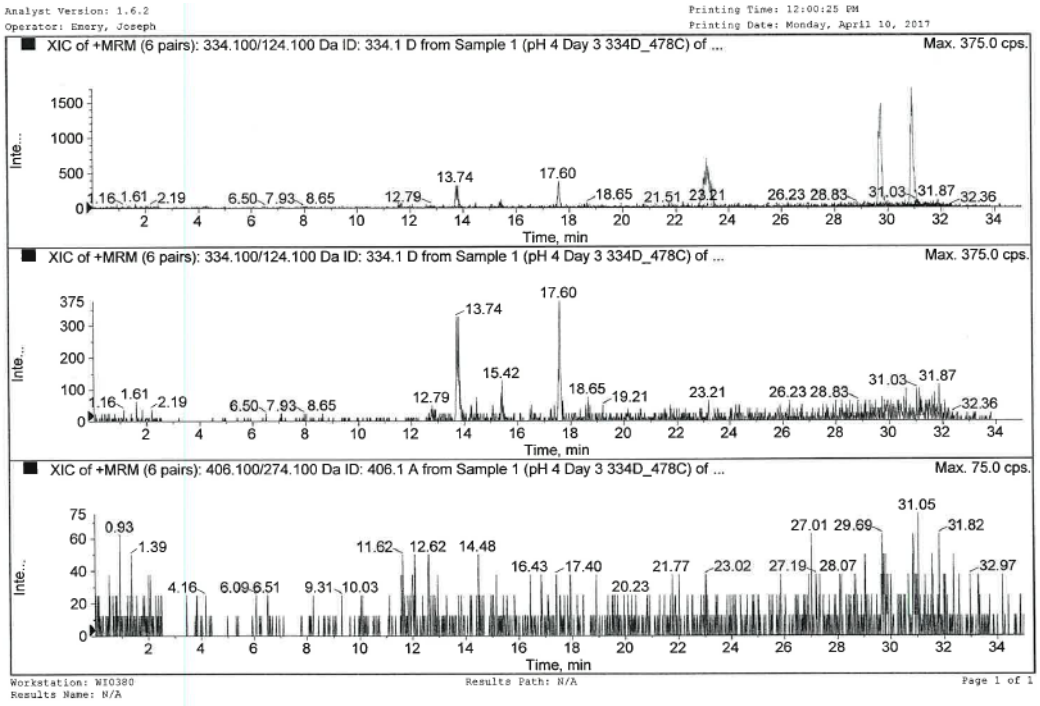


Figure 6: Mass Spectral Analysis for K32 Sample in pH 4 Buffer (MRM Analysis) from Hydrolysis Sample, Day 3 continued

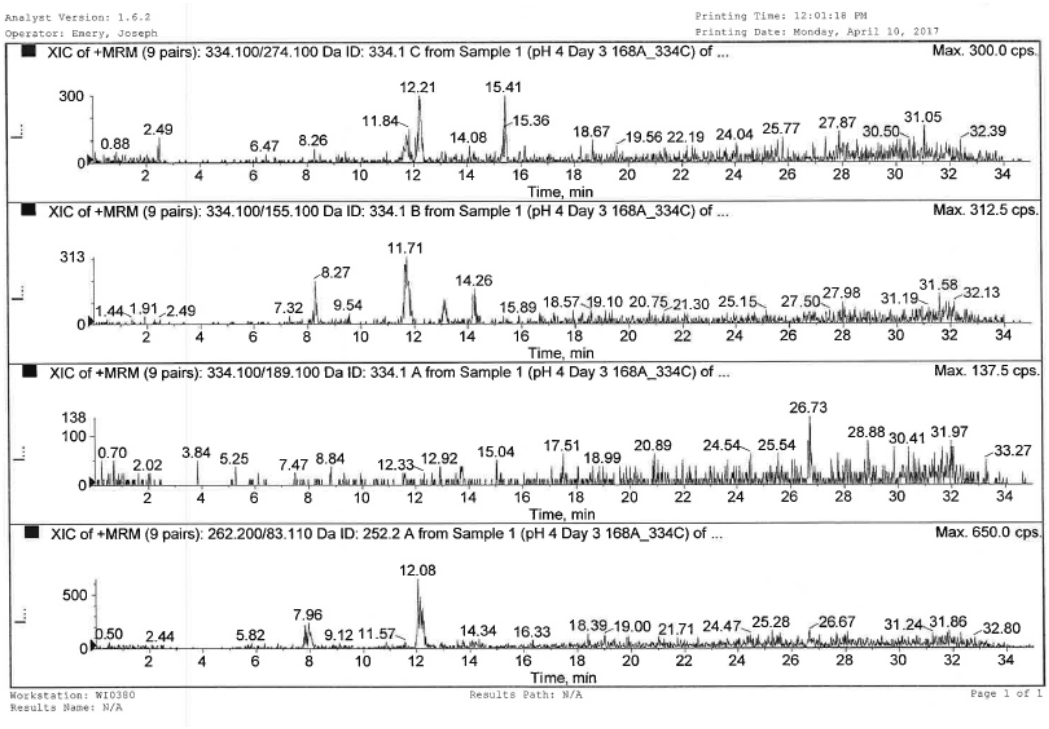


Figure 6: Mass Spectral Analysis for K32 Sample in pH 4 Buffer (MRM Analysis) from Hydrolysis Sample, Day 3 continued

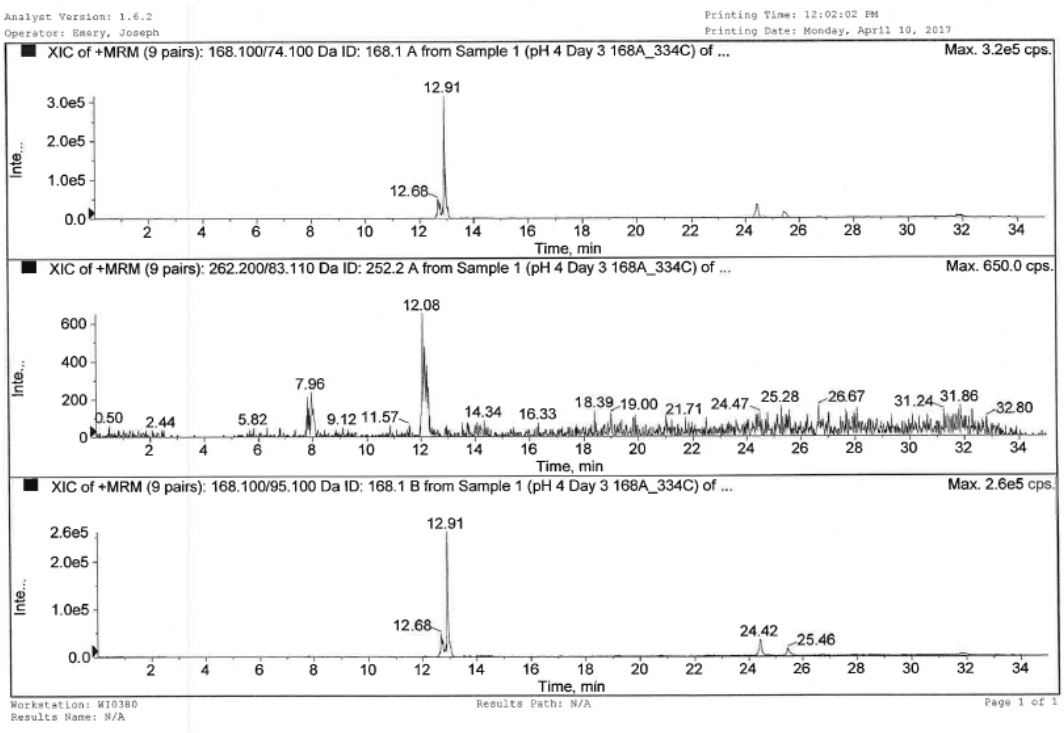
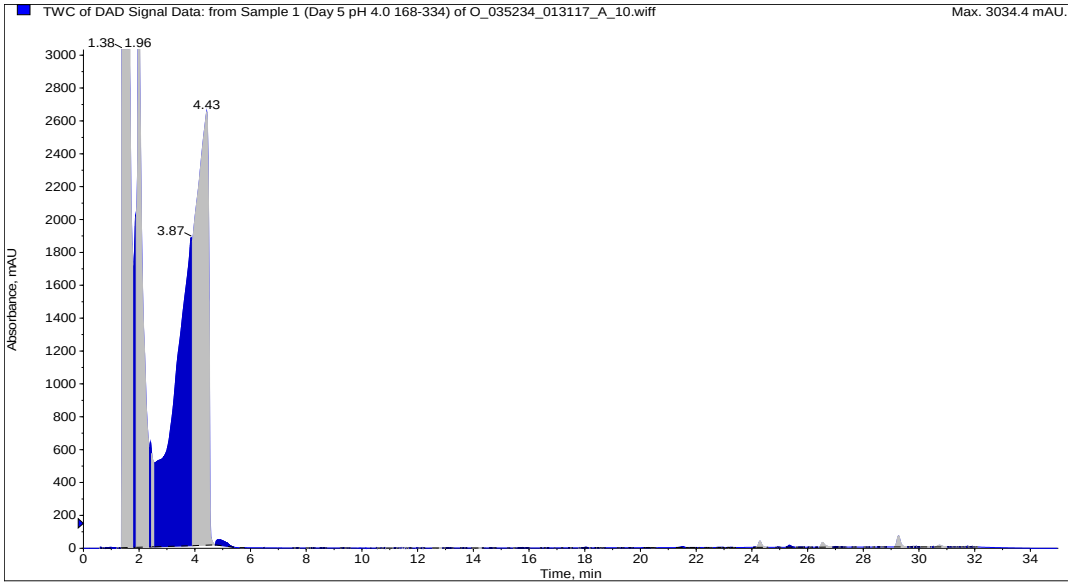


Figure 7: Representative LC-MS/UV Chromatogram of K32 Hydrolysis Sample in pH 4 Buffer, Day 5



Expanded 6-18 minutes

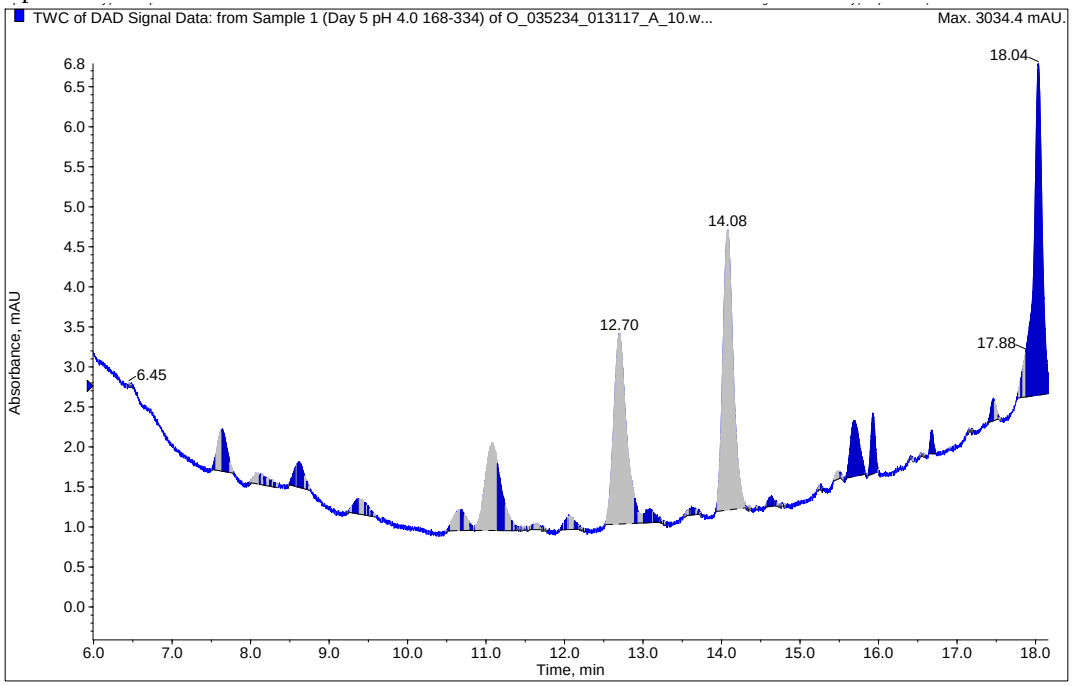


Figure 8: Mass Spectral Analysis for K32 Sample in pH 4 Buffer (MRM Analysis) from Hydrolysis Sample, Day 5

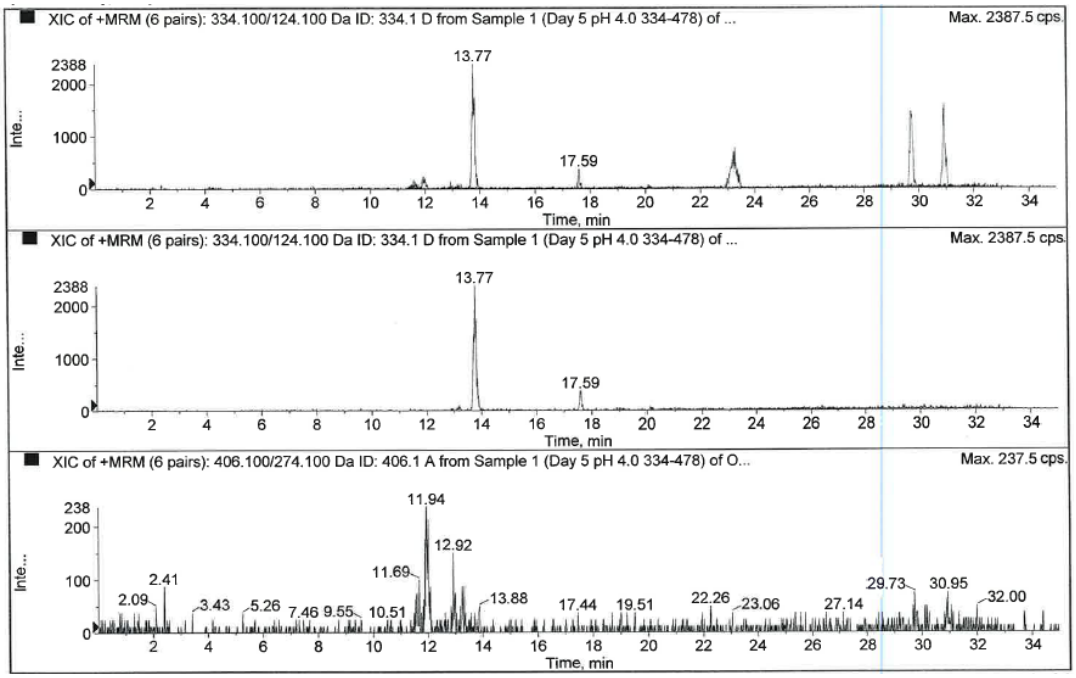


Figure 8: Mass Spectral Analysis for K32 Sample in pH 4 Buffer (MRM Analysis) from Hydrolysis Sample, Day 5 continued

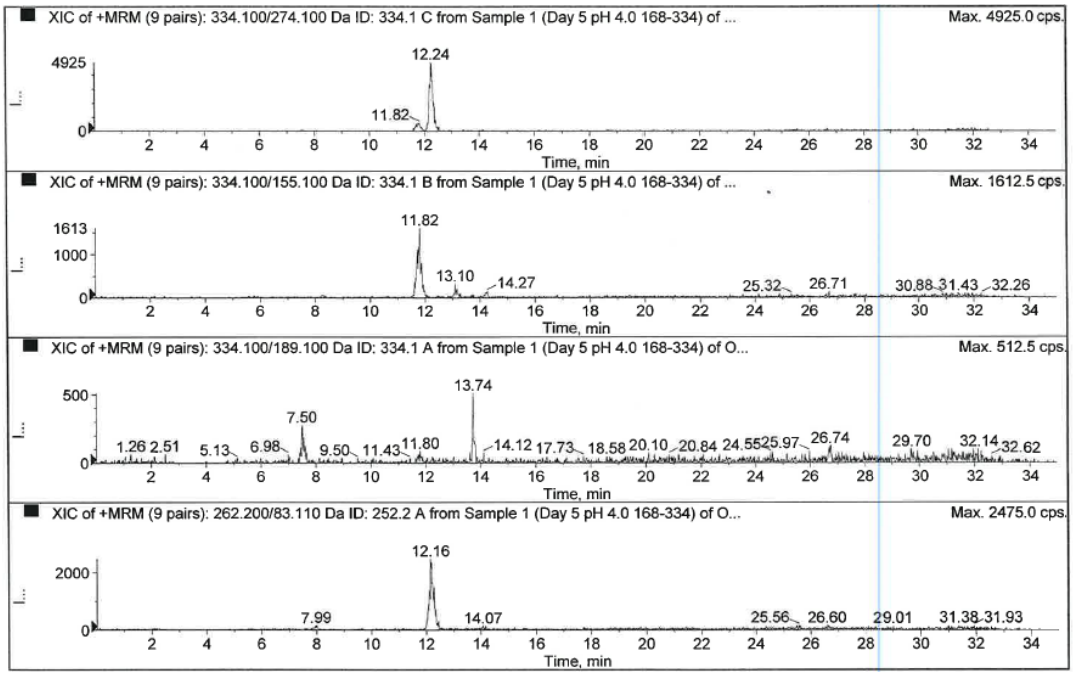


Figure 8: Mass Spectral Analysis for K32 Sample in pH 4 Buffer (MRM Analysis) from Hydrolysis Sample, Day 5 continued

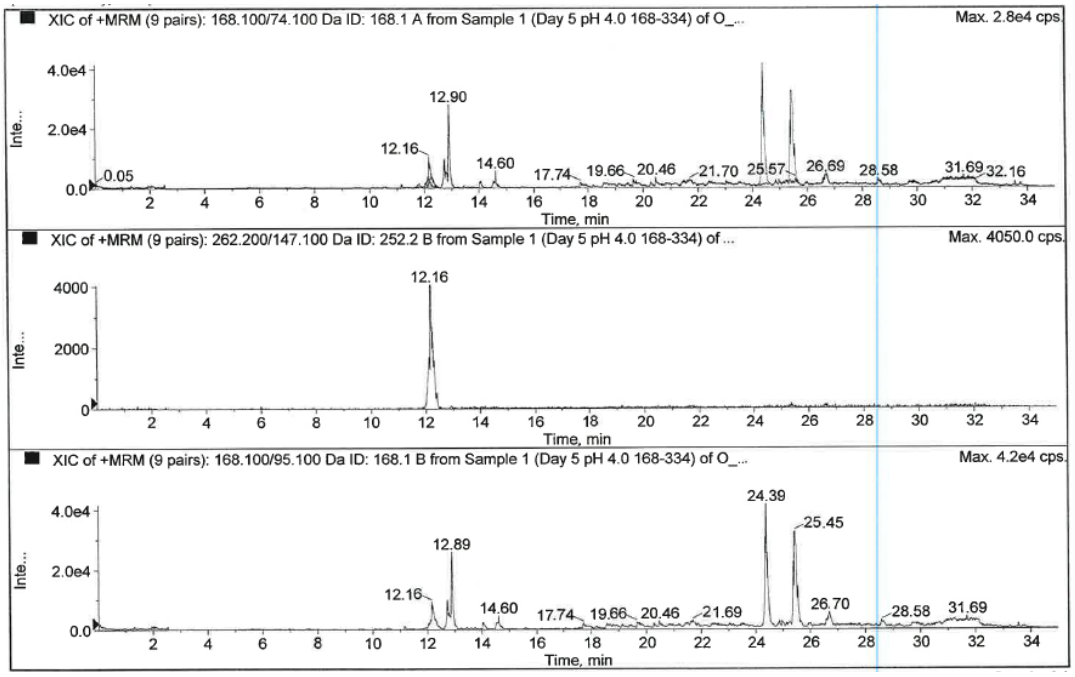
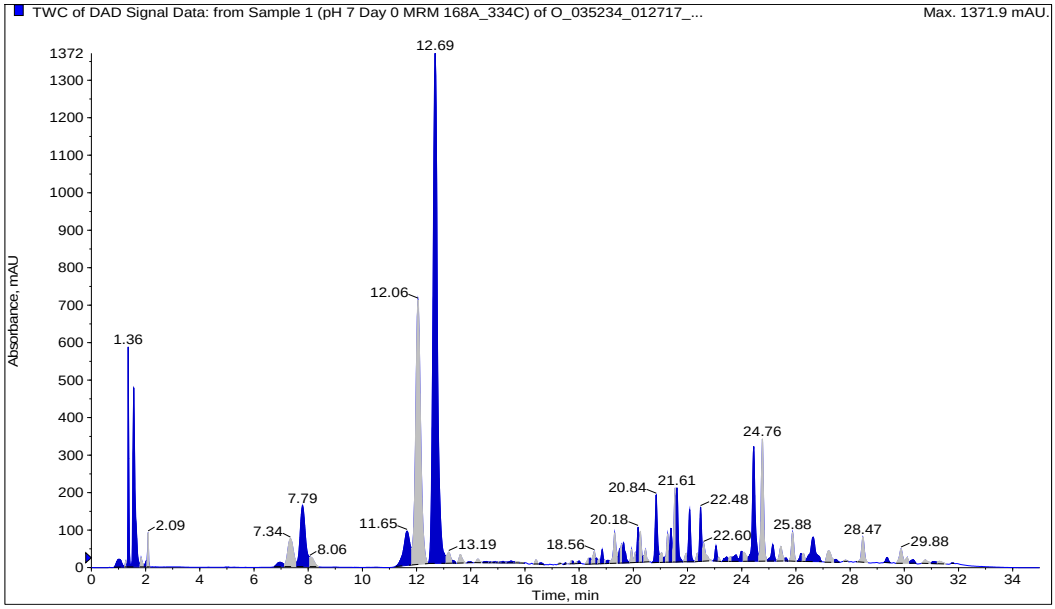


Figure 9: Representative LC-MS/UV Chromatogram of K32 Hydrolysis Sample in pH 7 Buffer, Day 0



Expanded 6 – 18 minutes

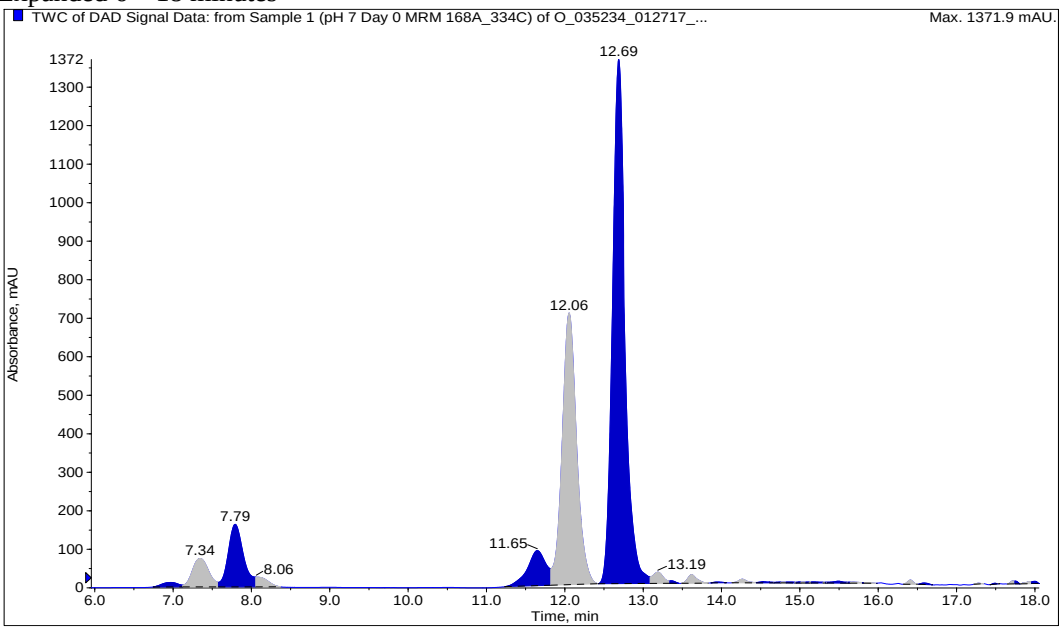


Figure 10: Mass Spectral Analysis for K32 Sample in pH 7 Buffer (MRM Analysis) from Hydrolysis Sample, Day 0

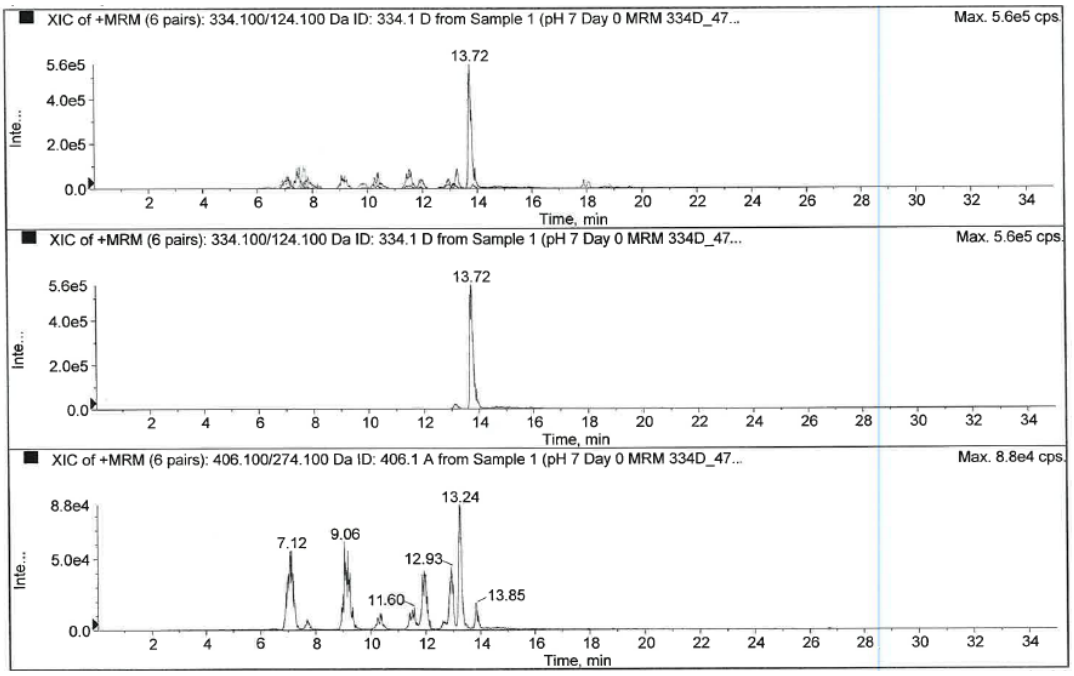


Figure 10: Mass Spectral Analysis for K32 Sample in pH 7 Buffer (MRM Analysis) from Hydrolysis Sample, Day 0 continued

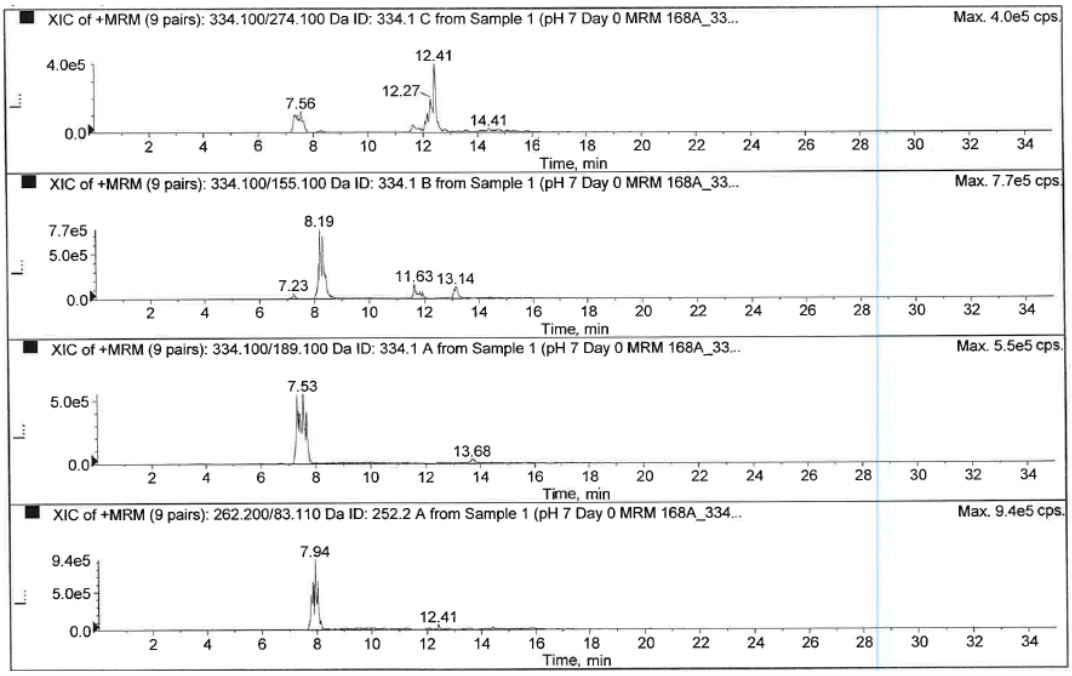


Figure 10: Mass Spectral Analysis for K32 Sample in pH 7 Buffer (MRM Analysis) from Hydrolysis Sample, Day 0 continued

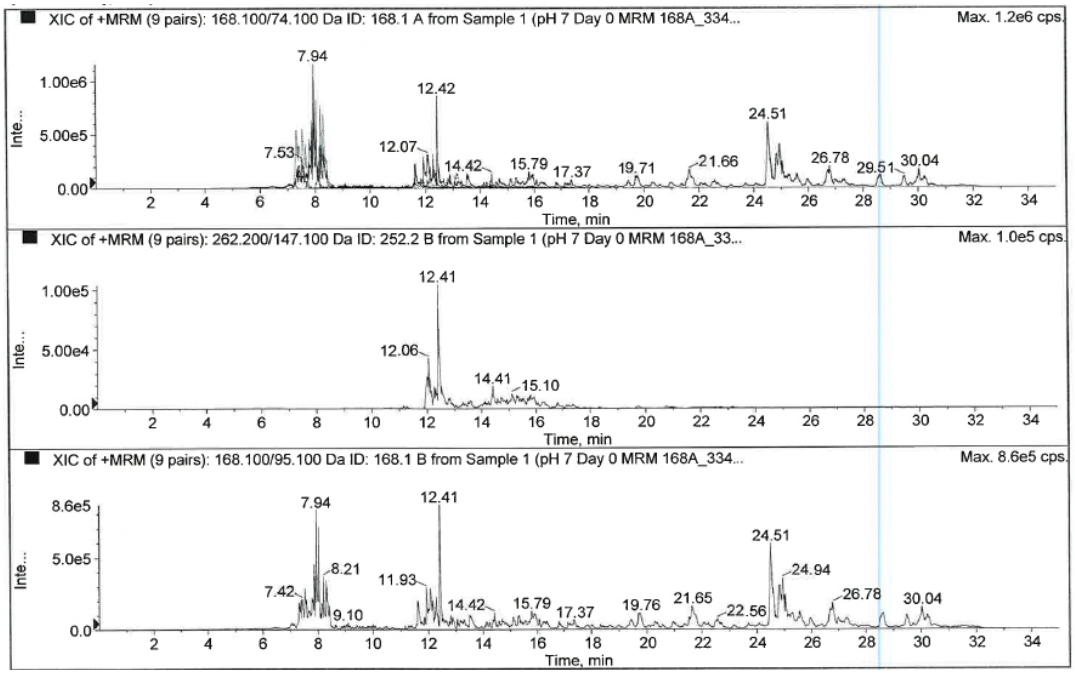
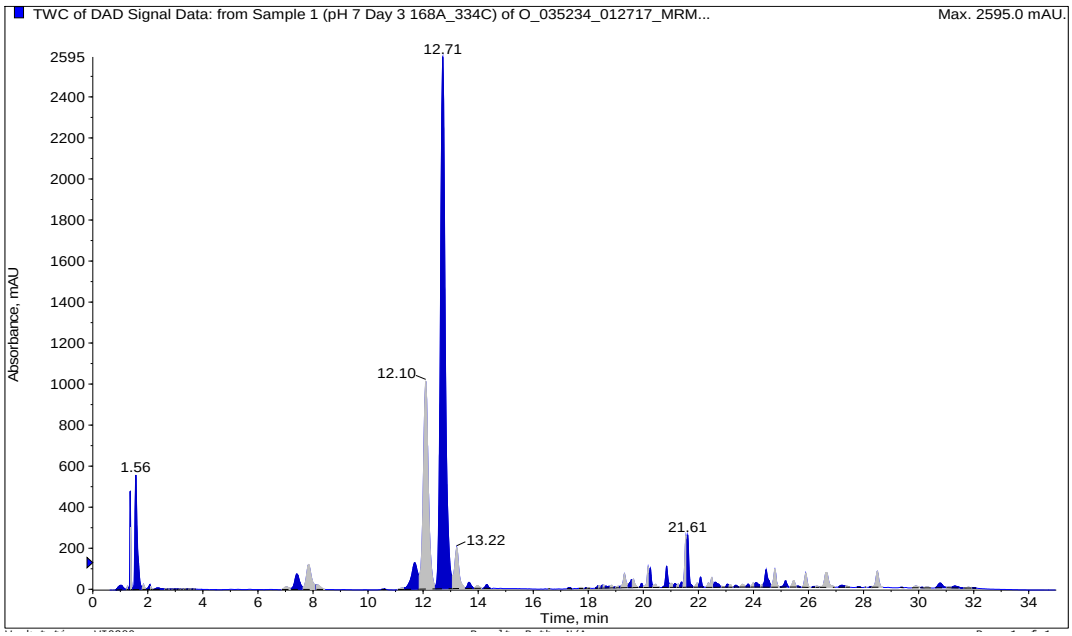


Figure 11: Representative LC-MS/UV Chromatogram of K32 Hydrolysis Sample in pH 7 Buffer, Day 3



Expanded 6- 18 minutes

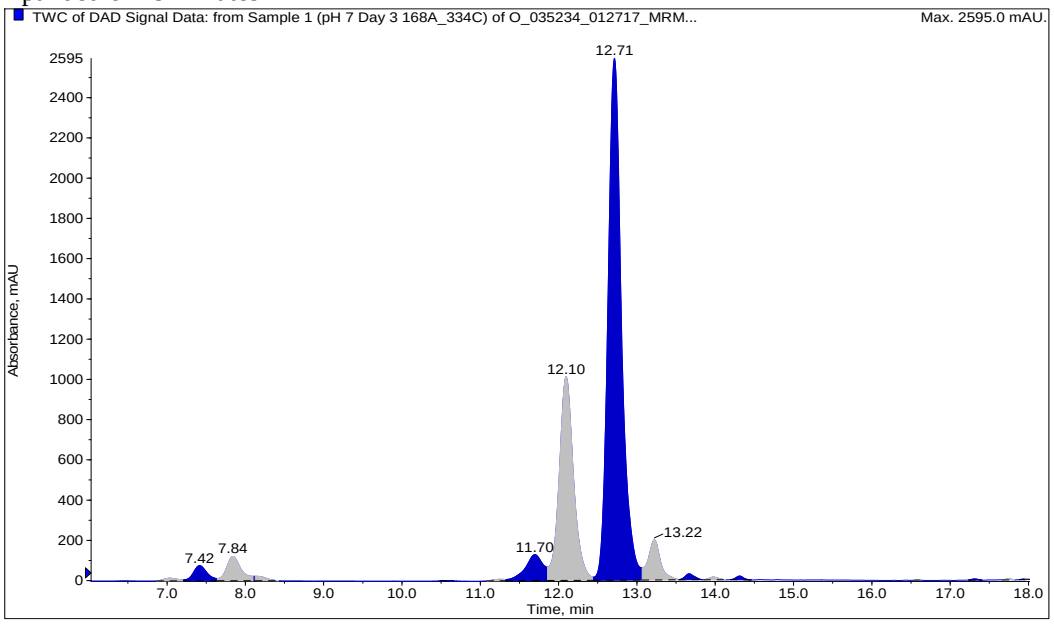


Figure 12: Mass Spectral Analysis for K32 Sample in pH 7 Buffer (MRM Analysis) from Hydrolysis Sample, Day 3

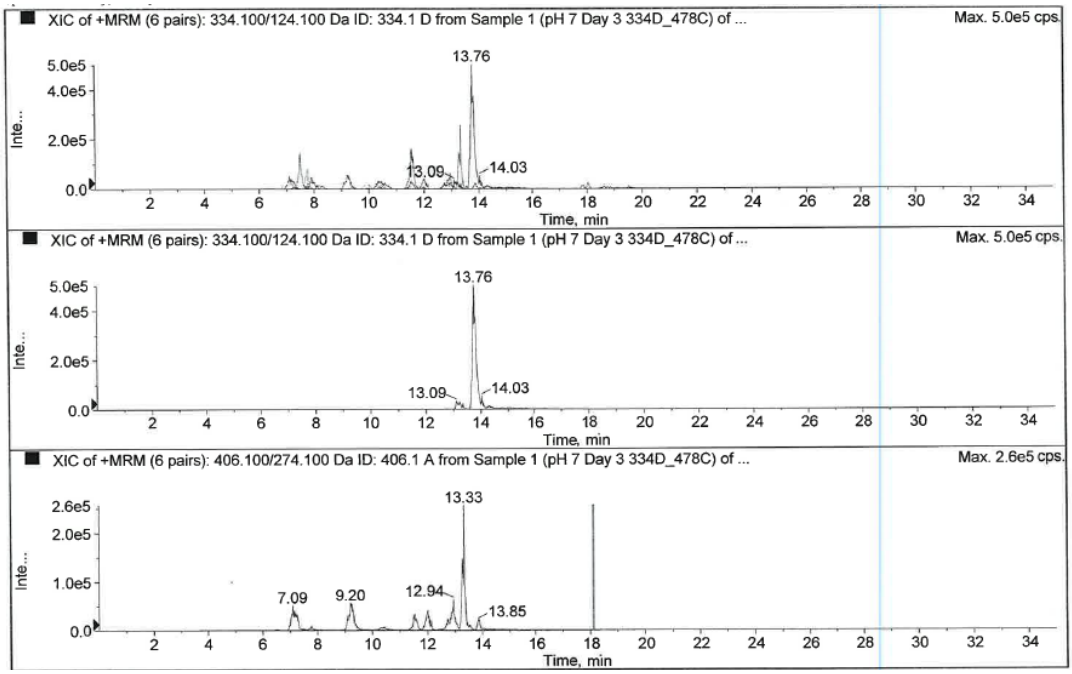


Figure 12: Mass Spectral Analysis for K32 Sample in pH 7 Buffer (MRM Analysis) from Hydrolysis Sample, Day 3 continued

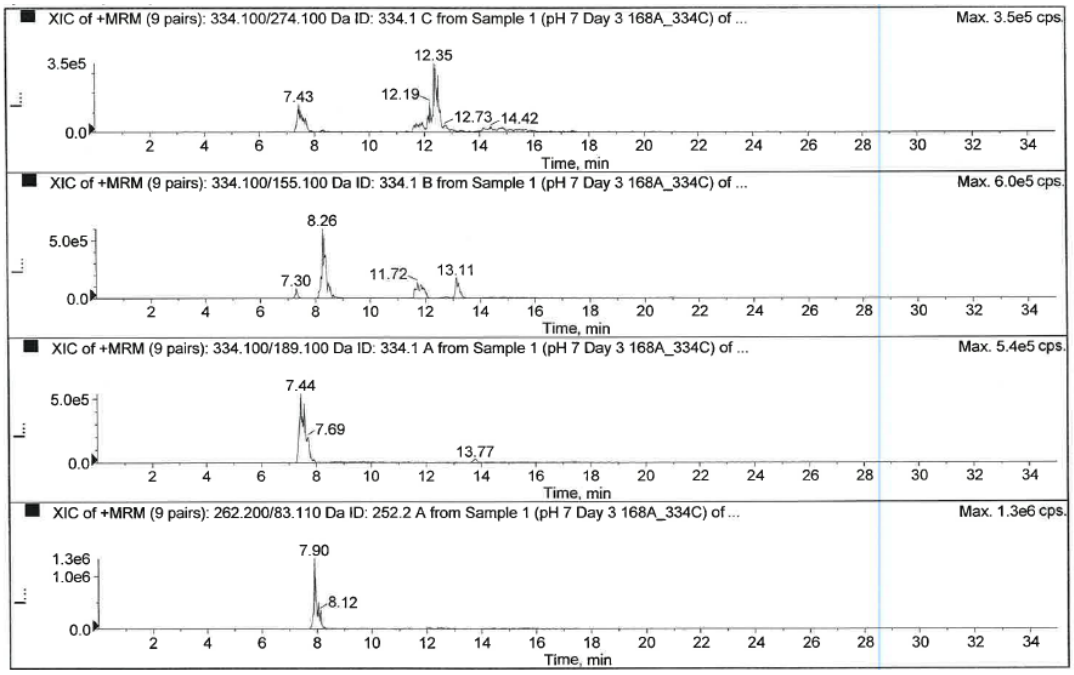


Figure 12: Mass Spectral Analysis for K32 Sample in pH 7 Buffer (MRM Analysis) from Hydrolysis Sample, Day 3 continued

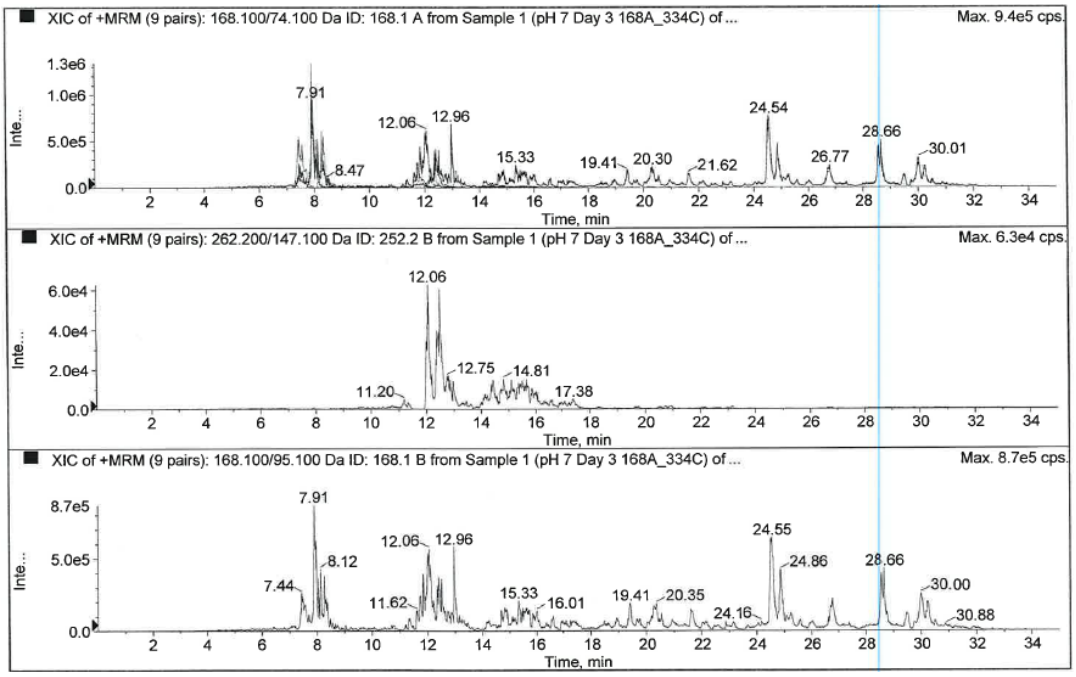
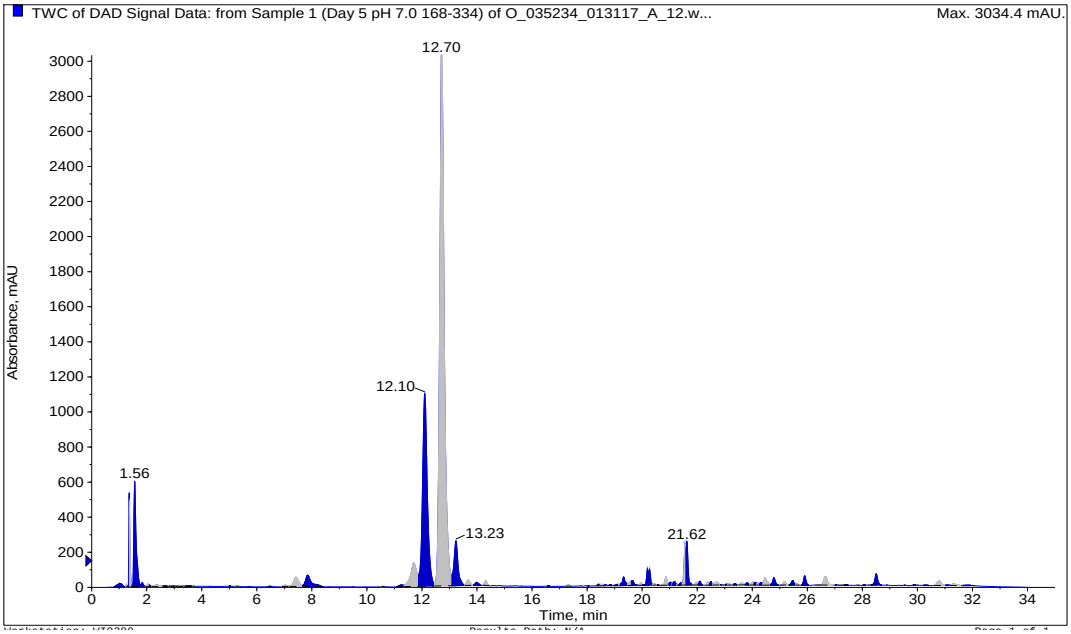


Figure 13: Representative LC-MS/UV Chromatogram of K32 Hydrolysis Sample in pH 7 Buffer, Day 5



Expanded 6- 18 minutes

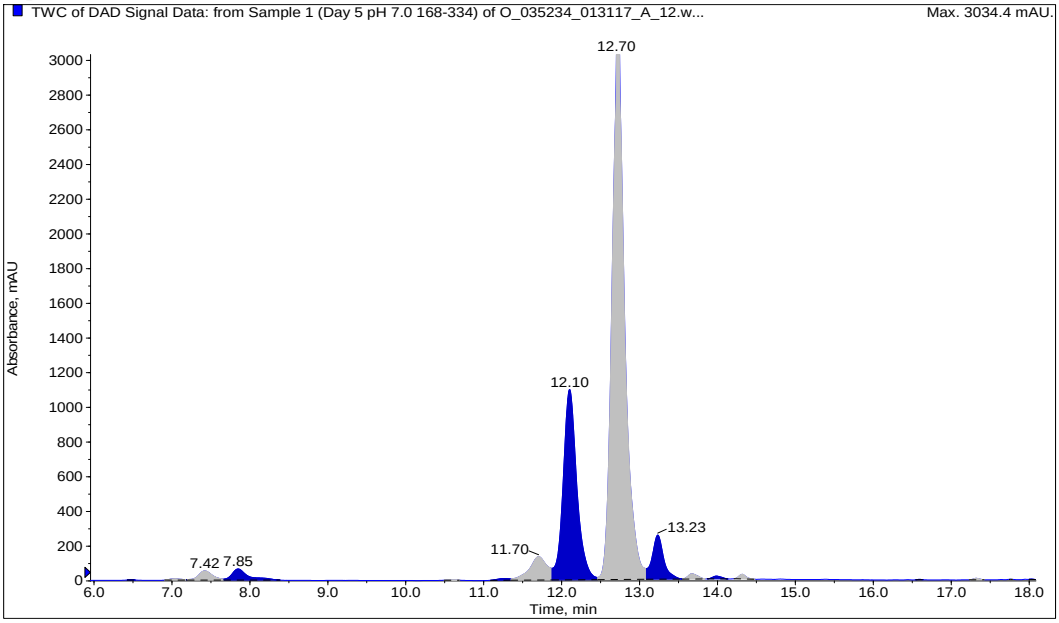


Figure 14: Mass Spectral Analysis for K32 Sample in pH 7 Buffer (MRM Analysis) from Hydrolysis Sample, Day 5

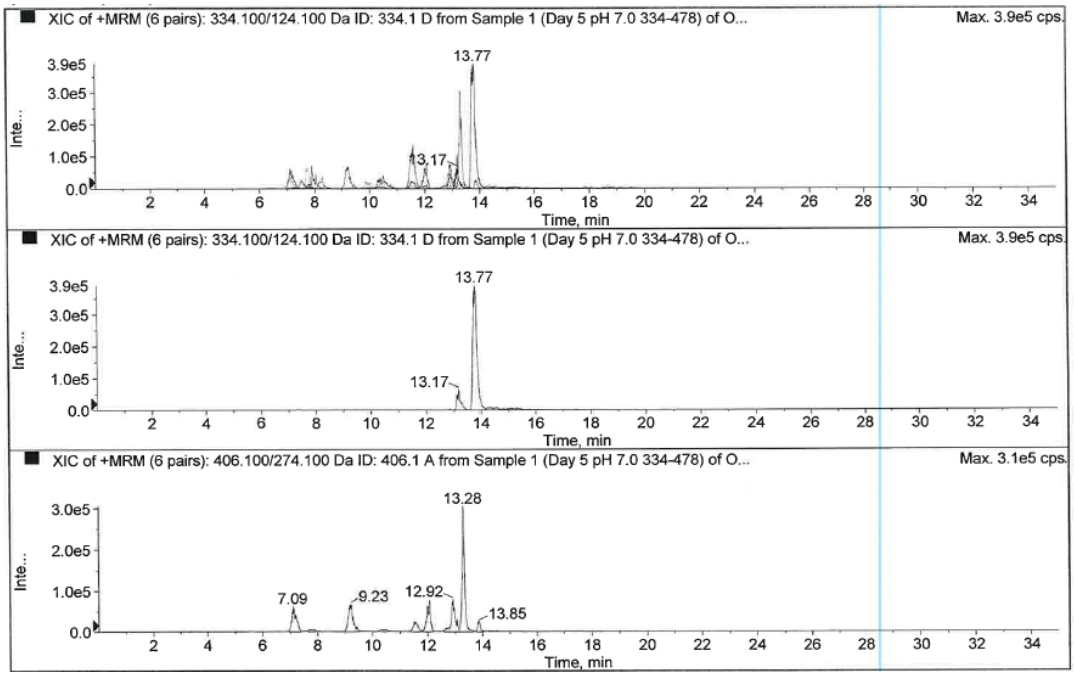


Figure 14: Mass Spectral Analysis for K32 Sample in pH 7 Buffer (MRM Analysis) from Hydrolysis Sample, Day 5 continued

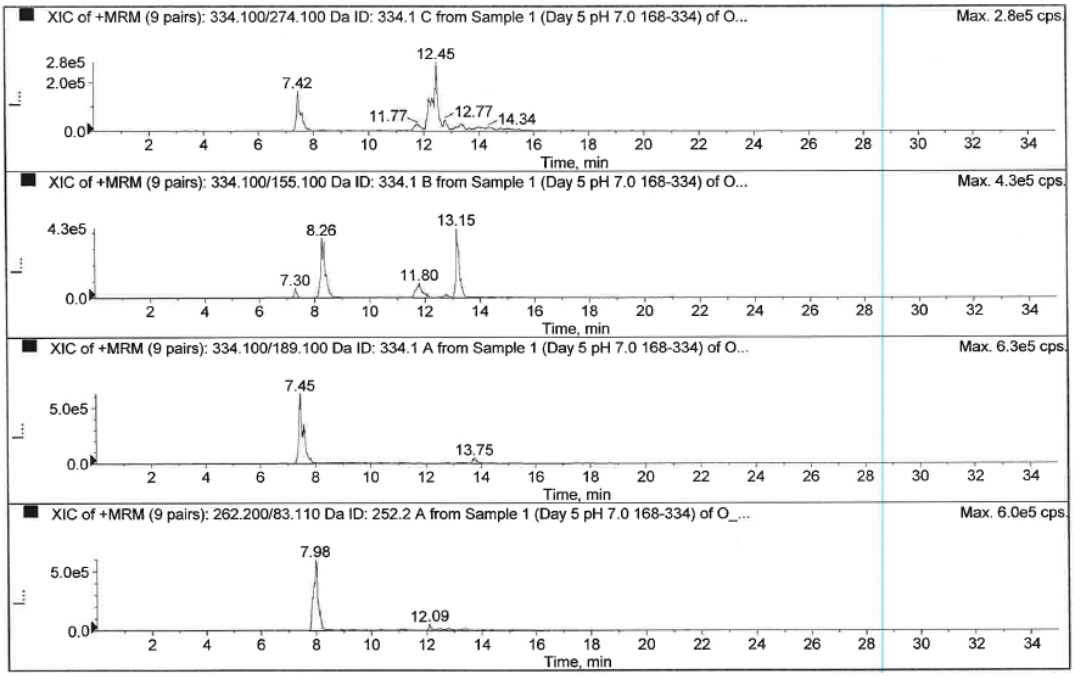


Figure 14: Mass Spectral Analysis for K32 Sample in pH 7 Buffer (MRM Analysis) from Hydrolysis Sample, Day 5 continued

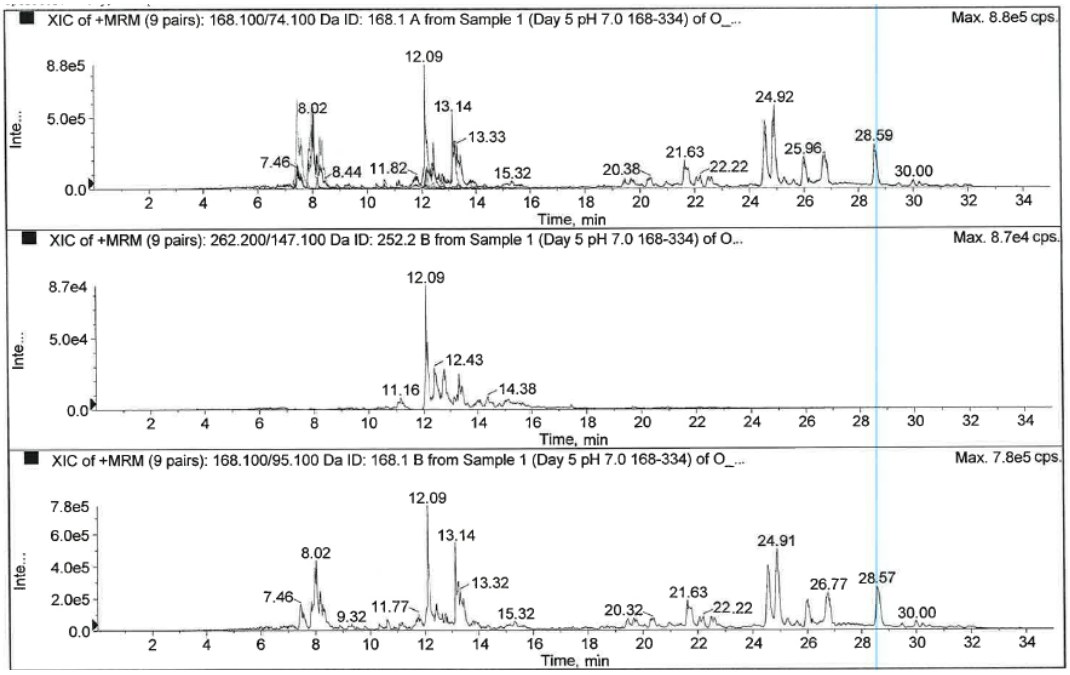
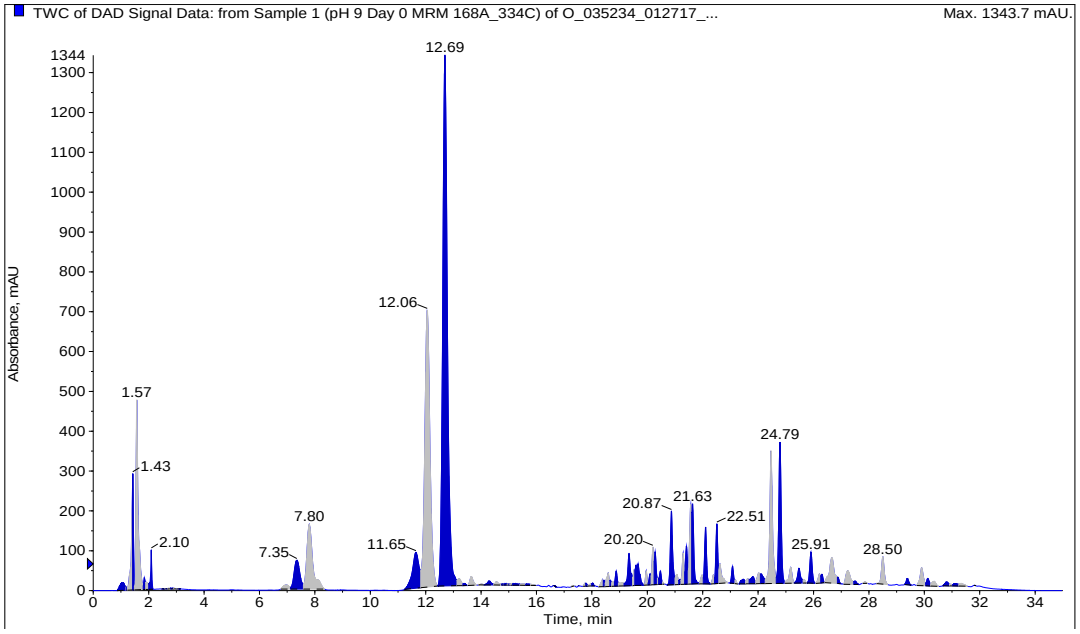


Figure 15: Representative LC-MS/UV Chromatogram of K32 Hydrolysis Sample in pH 9 Buffer, Day 0



Expanded 6-18 minutes

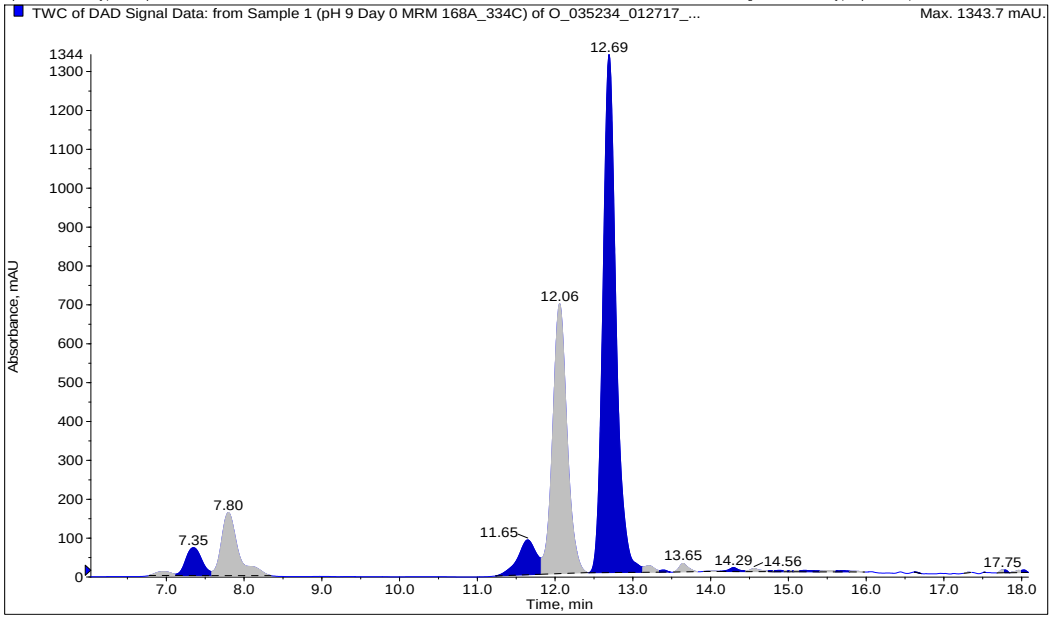


Figure 16: Mass Spectral Analysis for K32 Sample in pH 9 Buffer (MRM Analysis) from Hydrolysis Sample, Day 0

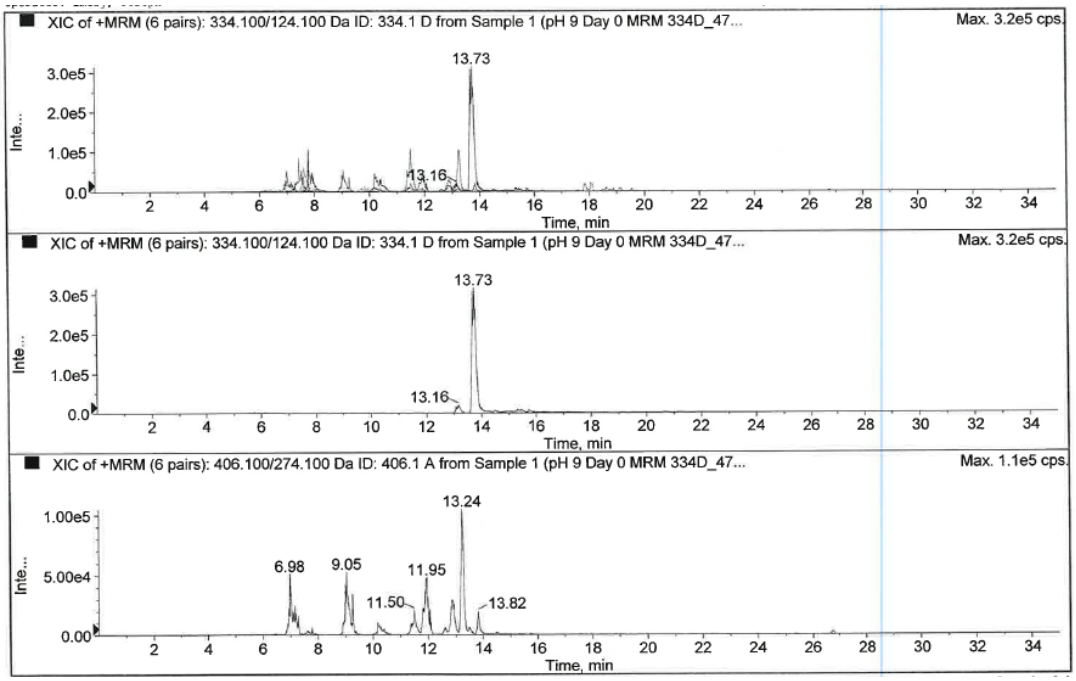


Figure 16: Mass Spectral Analysis for K32 Sample in pH 9 Buffer (MRM Analysis) from Hydrolysis Sample, Day 0 continued

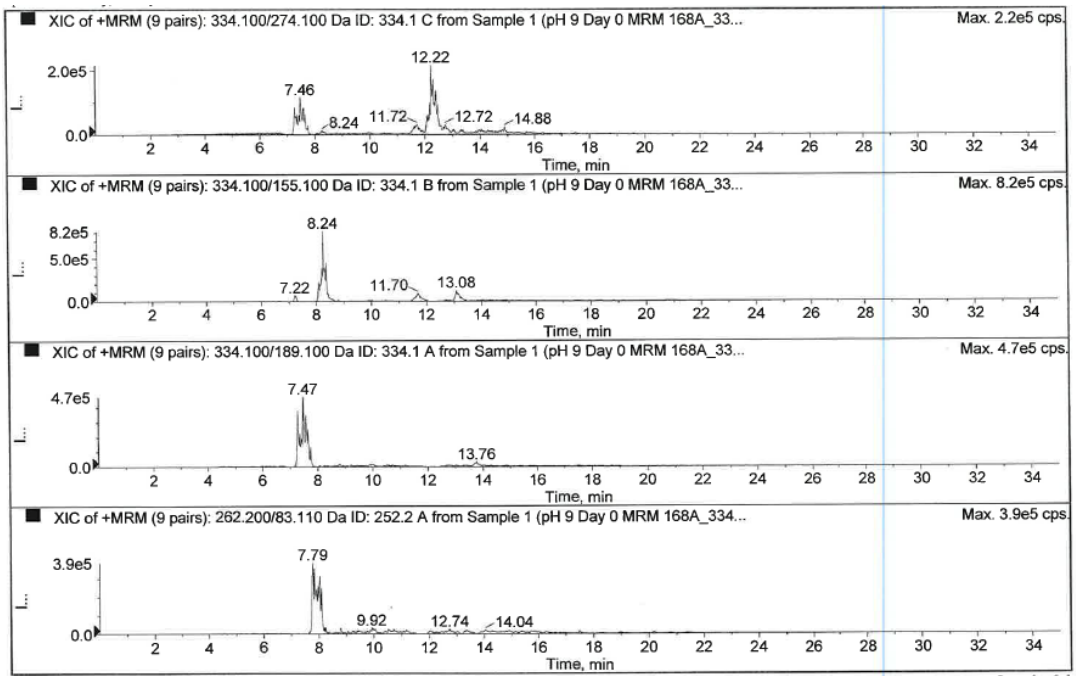


Figure 16: Mass Spectral Analysis for K32 Sample in pH 9 Buffer (MRM Analysis) from Hydrolysis Sample, Day 0 continued

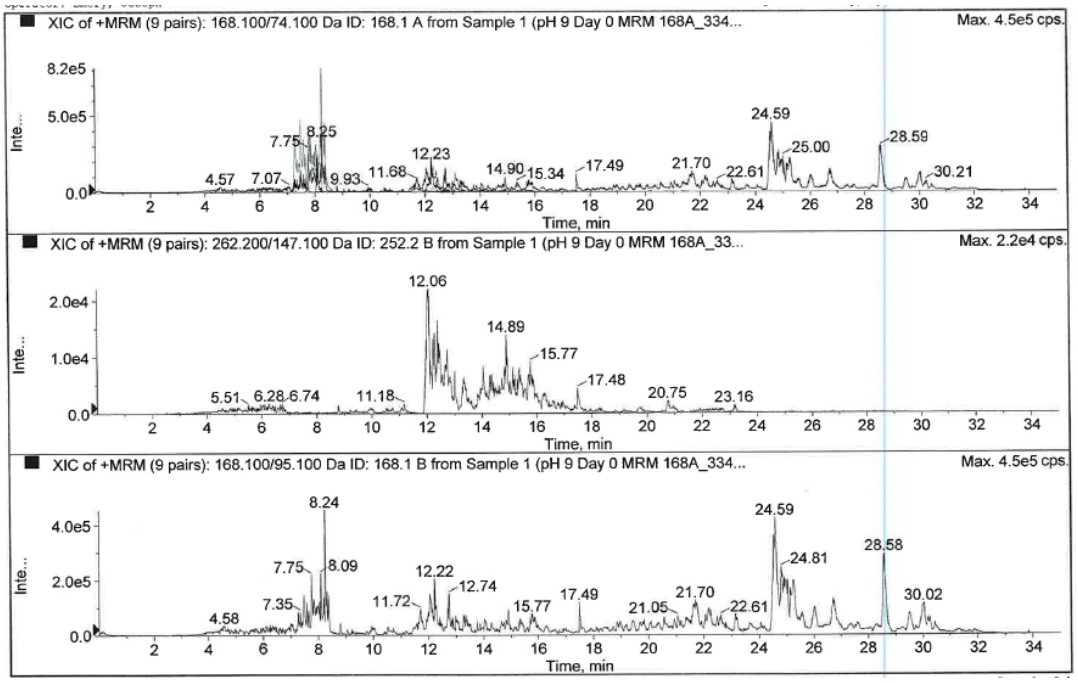
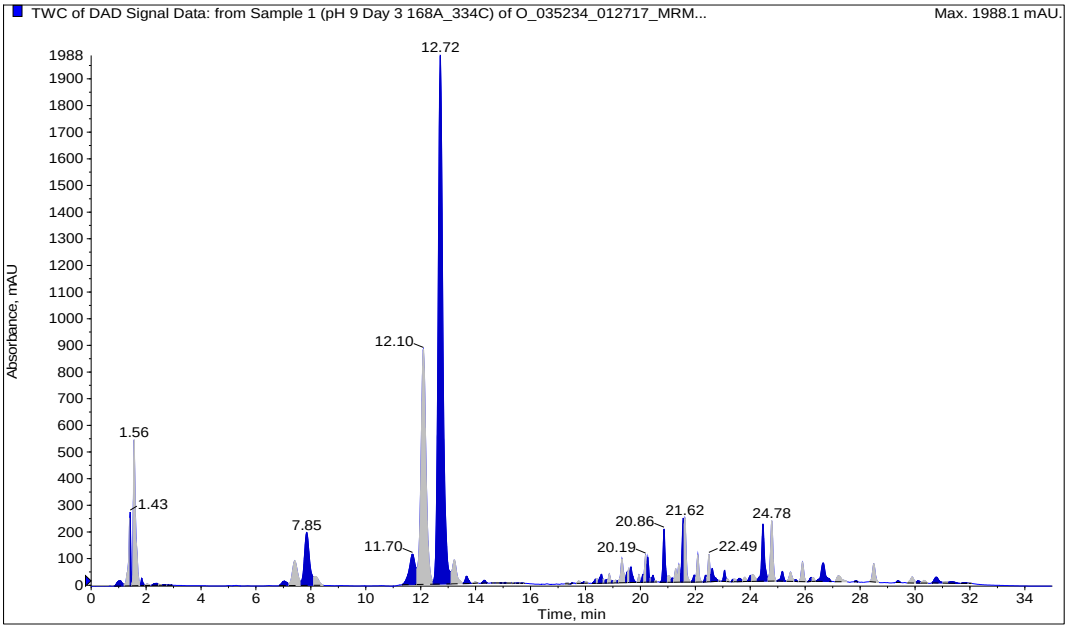


Figure 17: Representative LC-MS/UV Chromatogram of K32 Hydrolysis Sample in pH 9 Buffer, Day 3



Expanded 6-18 minutes

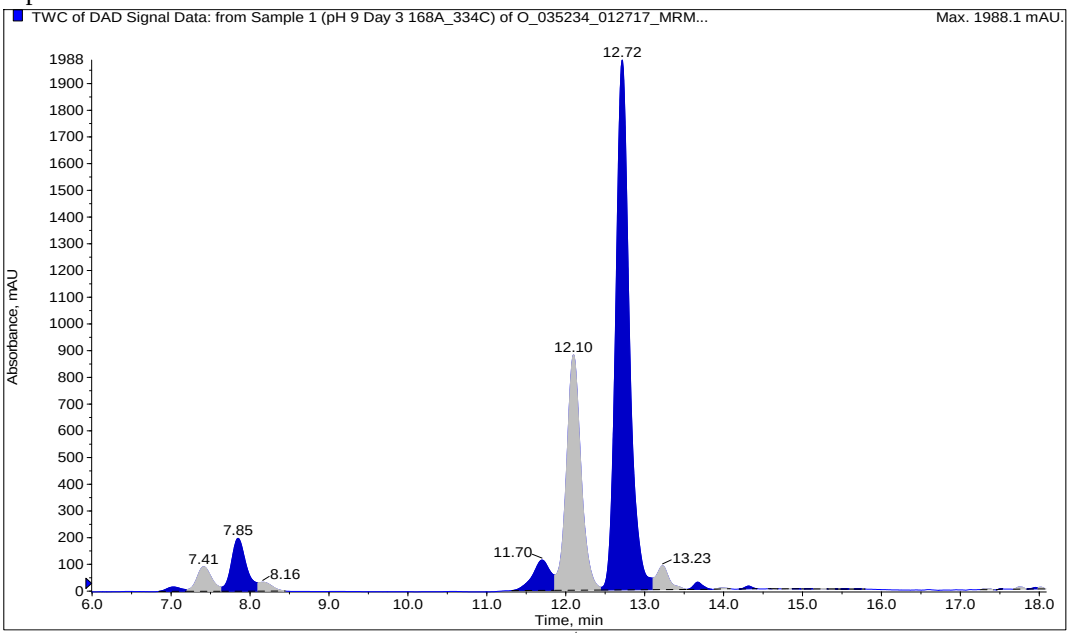


Figure 18: Mass Spectral Analysis for K32 Sample in pH 9 Buffer (MRM Analysis) from Hydrolysis Sample, Day 3

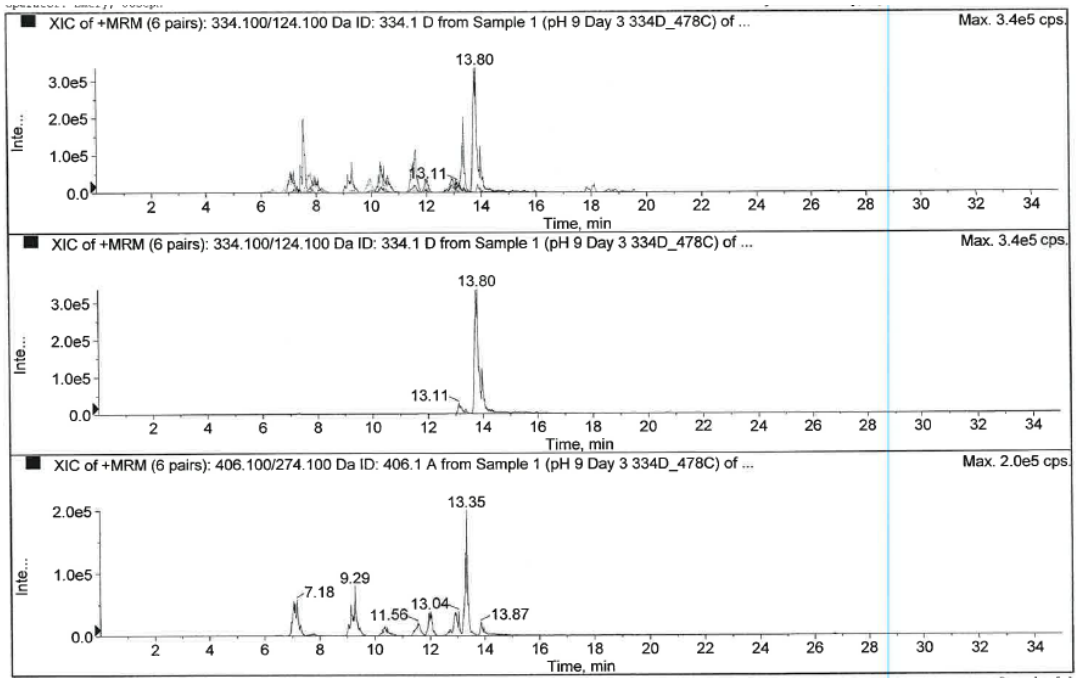


Figure 18: Mass Spectral Analysis for K32 Sample in pH 9 Buffer (MRM Analysis) from Hydrolysis Sample, Day 3 continued

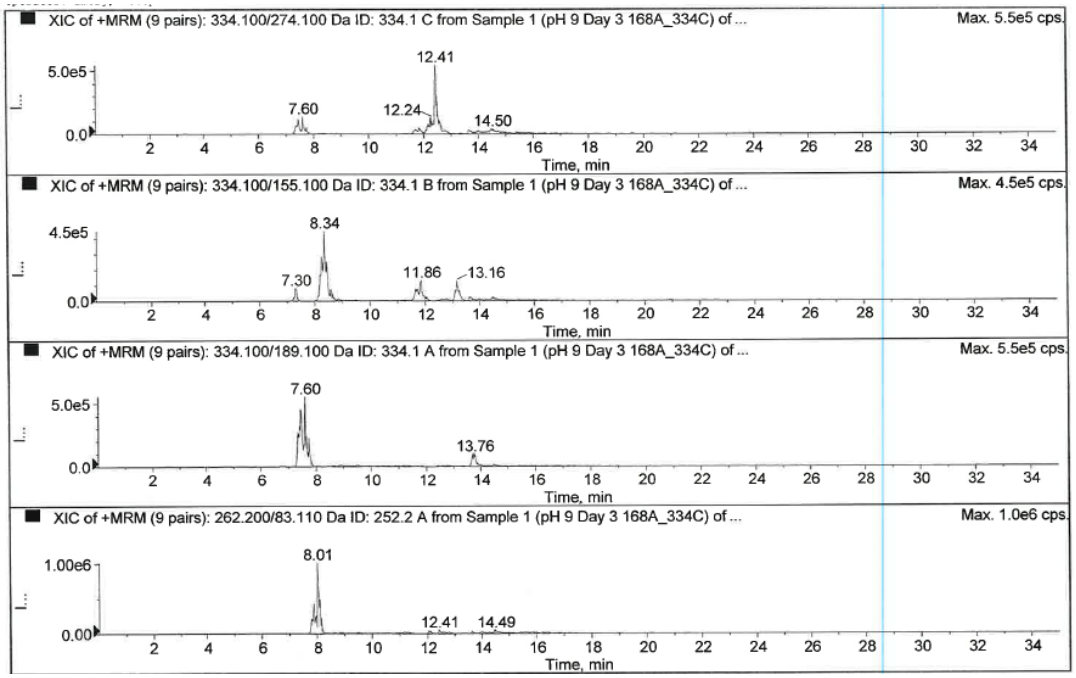


Figure 18: Mass Spectral Analysis for K32 Sample in pH 9 Buffer (MRM Analysis) from Hydrolysis Sample, Day 3 continued

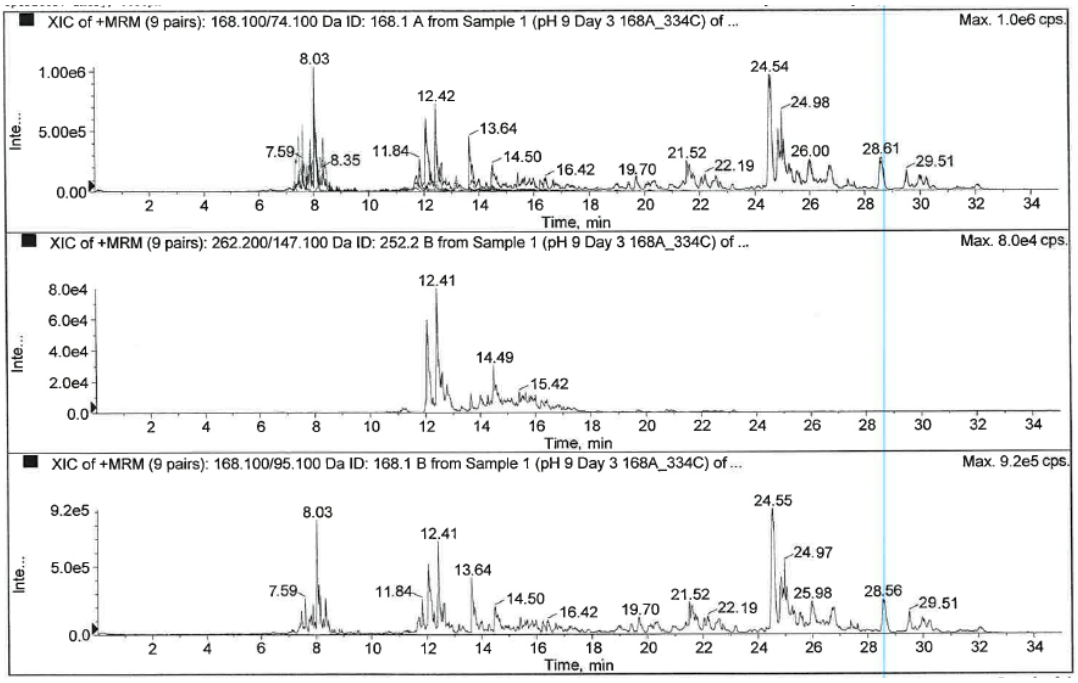
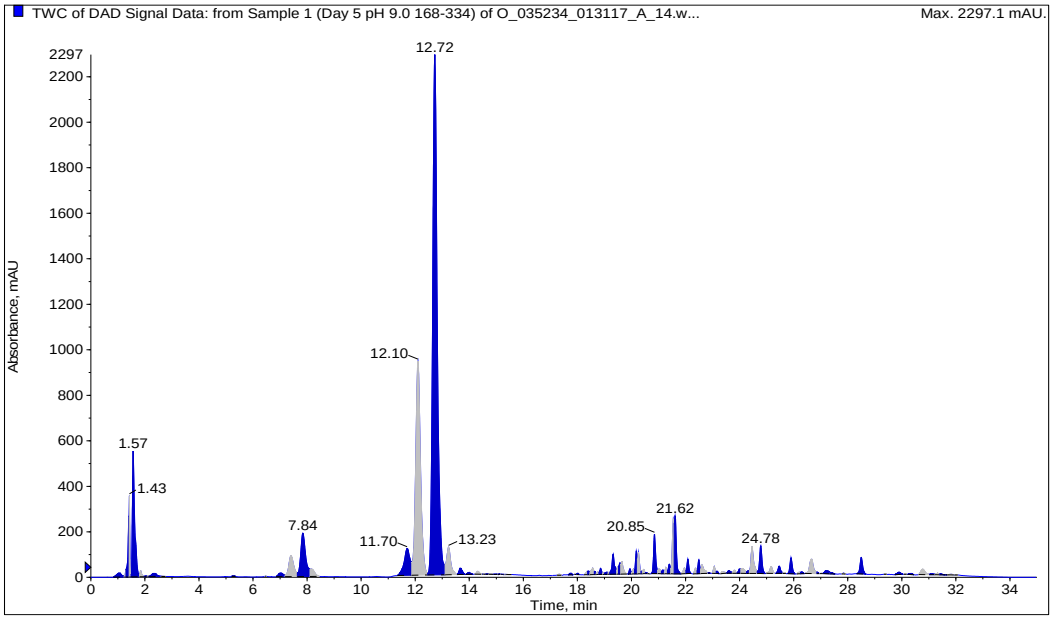


Figure 19: Representative LC-MS/UV Chromatogram of K32 Hydrolysis Sample in pH 9 Buffer, Day 5



Expanded 6-18 minutes

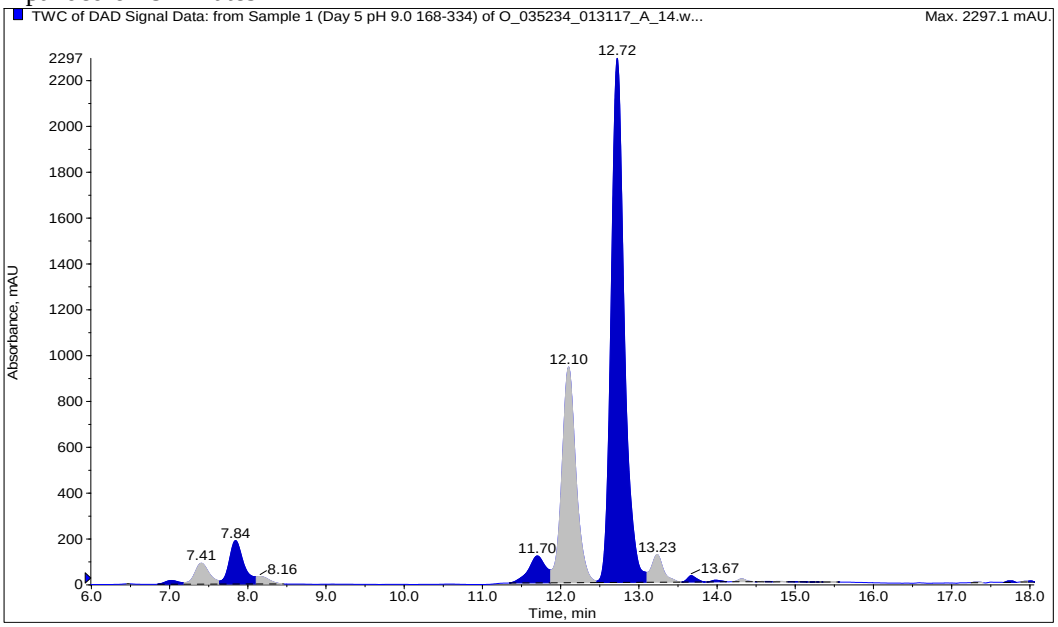


Figure 20: Mass Spectral Analysis for K32 Sample in pH 9 Buffer (MRM Analysis) from Hydrolysis Sample, Day 5

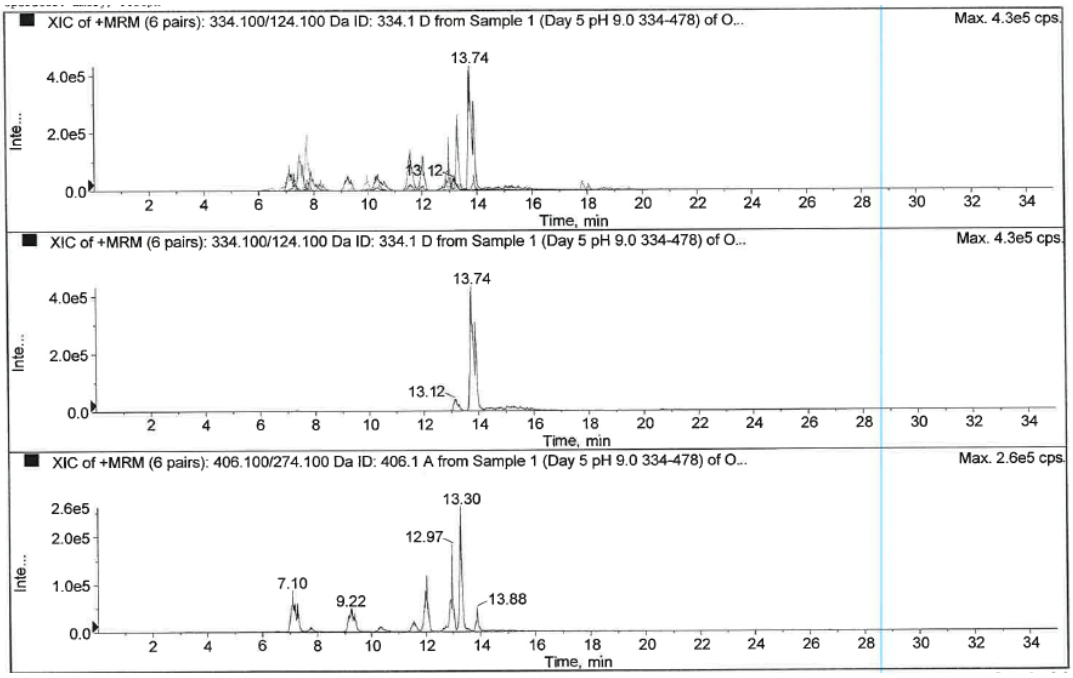


Figure 20: Mass Spectral Analysis for K32 Sample in pH 9 Buffer (MRM Analysis) from Hydrolysis Sample, Day 5 continued

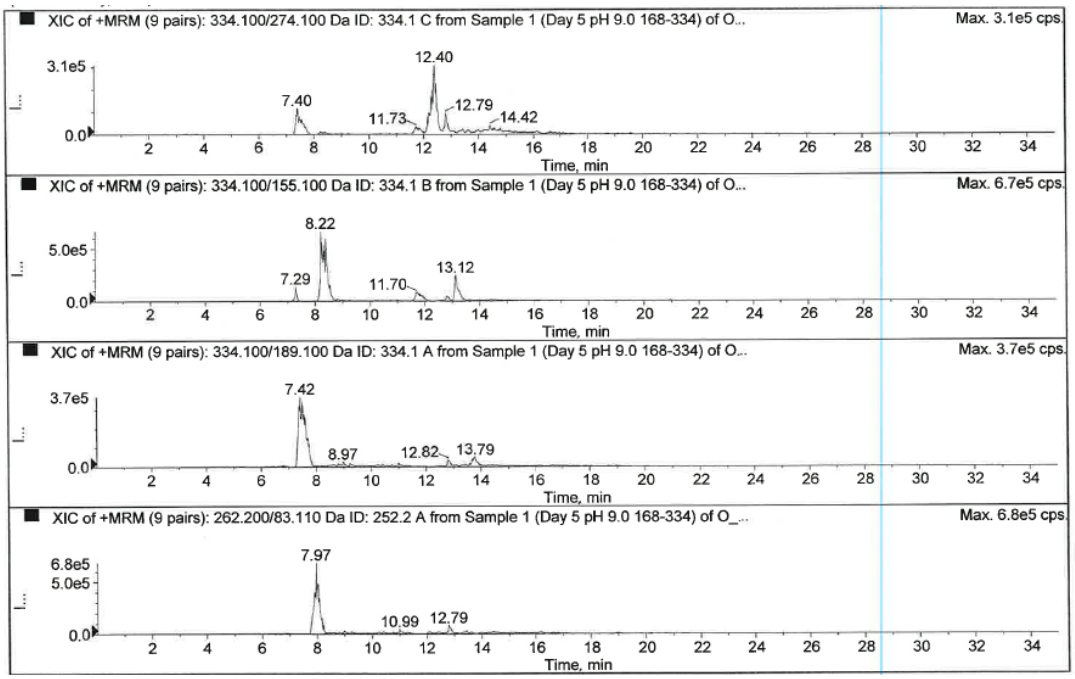


Figure 20: Mass Spectral Analysis for K32 Sample in pH 9 Buffer (MRM Analysis) from Hydrolysis Sample, Day 5 continued

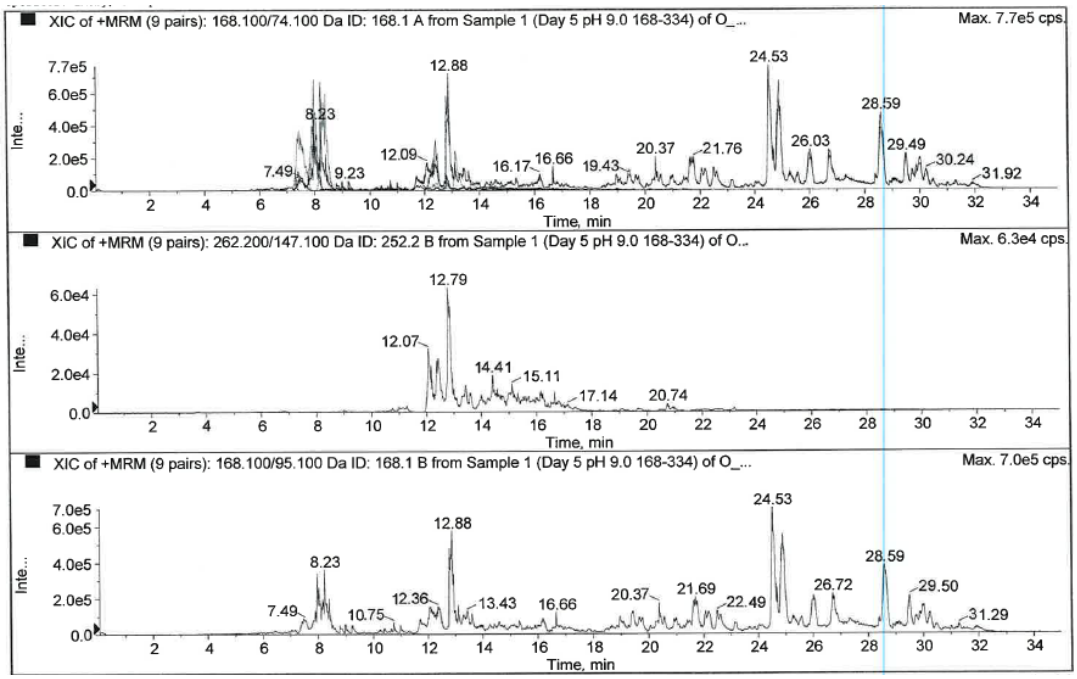


Figure 21: Component HPLC/UV Peak Area versus Time for pH 4 Buffer

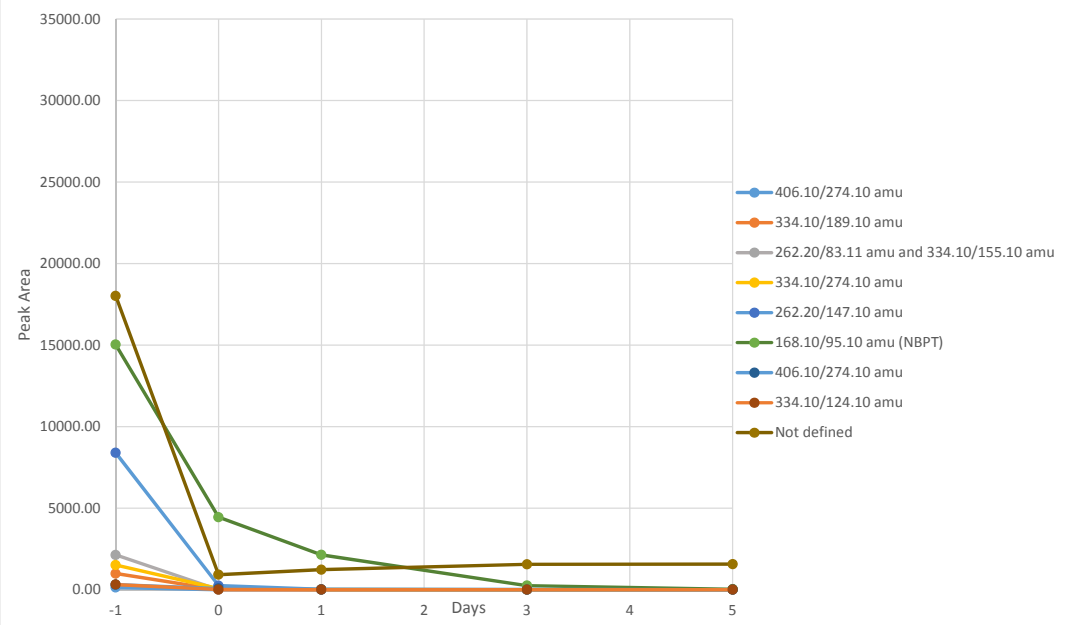


Figure 22: Component HPLC/UV Peak Area versus Time for pH 7 Buffer

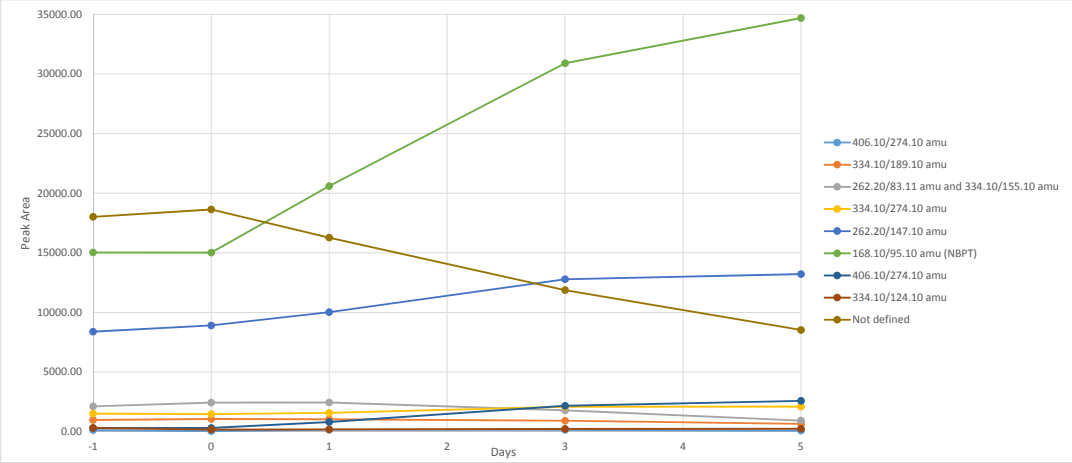
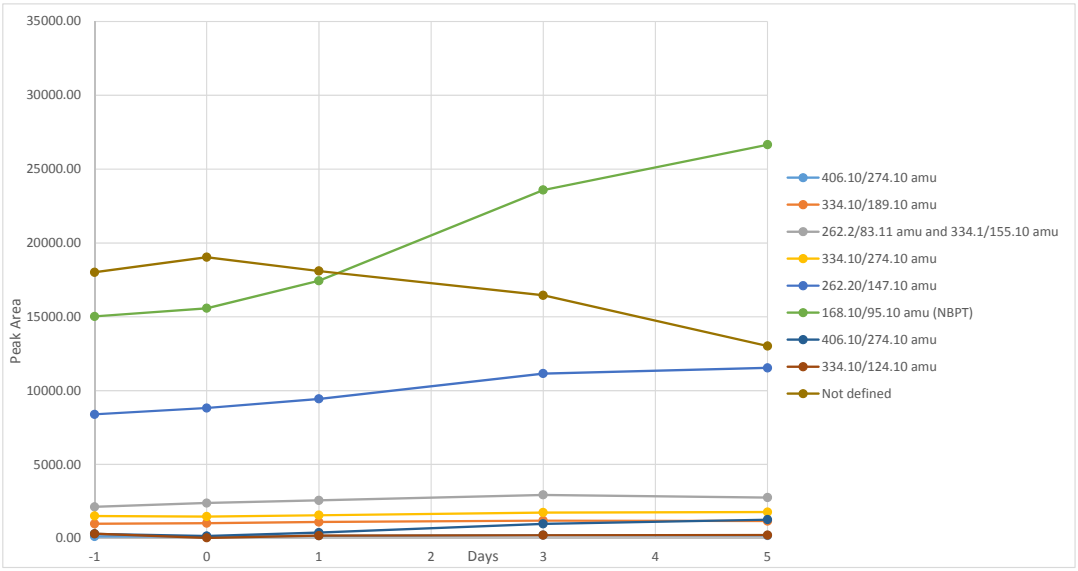


Figure 23: Component HPLC/UV Peak Area versus Time for pH 9 Buffer



APPENDIX A

Certificate of Analysis for K32 Lot 55700-30-13

CERTIFICATE OF ANALYSIS

K32

Batch/Lot No: 55700-30-13

Ricerca Sample Code: CS 22050

Test	Test Method	Test Result
Active Ingredient Content and Identification	HPLC-UV TAN and LC-MS	See Table Below
Phosphorus Content	ICP	12.4%
Identification and Relative Quantification	31P-NMR	See Table Below
N-(n-butyl) thiophosphoric triamide (NBPT) Content	HPLC	20.04%
Conditions:	Refrigerate for prolonged storage (2-8 °C); may be handled at room temperature	
Study Initiation Date:	November 1, 2016	
Study Completion Date:	November 30, 2016	

Active ingredient content by identification of peak using LC-UV TAN and LC-MS

Component	Observed Retention Time (minutes)	Observed %Area	Expected Molecular Mass	Observed molecular mass
1	6.58	0.2	478 and 406 $[M+Na]^+$	406.1 $[M+Na]^+$
2	6.98	1.9	334 $[M+Na]^+$	334.1 $[M+Na]^+$
3	7.48	4.2	262 $[M+Na]^+$	262.1 $[M+Na]^+$
3b	7.76	0.4		334.1 $[M+Na]^+$
4	10.83	0.5	406 $[M+Na]^+$	406.1 $[M+Na]^+$
5	11.06	3.1	334 $[M+Na]^+$	334.1 $[M+Na]^+$
6	11.48	17.5	262 $[M+Na]^+$	262.1 $[M+Na]^+$
7	12.16	30.2	190 $[M+Na]^+$	168.1 $[M+H]^+$
8	12.62	0.2	406 $[M+Na]^+$	406.1 $[M+Na]^+$
9	12.89	0.4	334 $[M+Na]^+$	334.1 $[M+Na]^+$
10	13.55	0.1	252 $[M+Na]^+$	274.1 $[M+Na]^+$
11	17.4 to 30.0	41.1	Not Defined, mixture	Not Defined, mixture

Identification and Relative Quantification using ^{31}P-NMR

3.53 wt% K32	Chemical shifts (ppm)	NMR integral	Concentration (mM/g)*	Relative Ratio (to NBPT)
K32-a	[-0.22 - -0.61]	0.12	0.01	0.01
K32-b	[53.37 - 52.91]	0.33	0.03	0.02
K32-c	[55.73 - 55.43]	0.11	0.01	0.01
K32-d	[58.15 - 57.85]	0.15	0.01	0.01
NBPT	[59.80 - 59.49]	14.42	1.10	1.00
K32-e	[60.07 - 59.80]	7.83	0.60	0.54
K32-f	[60.31 - 60.07]	9.55	0.73	0.66
K32-g	[61.65 - 60.54]	5.48	0.42	0.38
L32-h	[64.97 - 63.38]	8.97	0.69	0.62
Total	[0.00 - 70.00]	46.96	3.60	

**mMol of each compound in per gram of K32*

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CERTIFICATE OF ANALYSIS

Penny Miner, Study Director
AgChem Product Development

November 30, 2016
Date

Phillip Cassidy, Management
AgChem Product Development

November 30, 2016
Date

1. The objective of this study was to determine the active ingredient content, phosphorus content and mass spectra of K32, to be used as a test, reference or control substance in a study.
2. This study was conducted in accordance with the Good Laboratory Practice Standard, 40 CFR Part 160.135 (b).
3. No deviations occurred from GLP regulations, the protocol, and relevant SOPs.
4. Data for this Certificate of Analysis is archived at the address below under Project Number 035469.
5. Only descriptive statistics were used.

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